Bijvoet Center

for Biomolecular Research

Progress Report 2014-2015

"Discovering the Molecular Basis of Life"

Universiteit Utrecht

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Introduction

Dear colleagues,

It is our honour and pleasure to present you the progress report of the Bijvoet Center for Biomolecular Research over the years 2014 and 2015. As you will see, our Center has continued to be successful in the ever changing scientific and political climate in the Netherlands.

Just five years ago, the Bijvoet Center for Biomolecular Research redefined its mission to "discovering the Molecular Basis of Life". In the last two years, we have seen the research in the center has been moving towards understanding molecular aspects of life at increasing levels of complexity, all the way towards intact, living cells. By expanding into new research directions, we have been able to consolidate our leading position in the molecular life sciences and structural biology. This has also resulted in numerous awarded grants and the addition of new expertise to the Center.

Early 2014, prof. Casper Hoogenraad and prof. Anna Akhmanova of the Department of Biology became associate members of the Bijvoet Center. In the same year, prof. Geert-Jan Boons, a glycomics and chemical biology expert was appointed, jointly with the Utrecht Institute for Pharmaceutical Sciences, as a new professor at Utrecht University. This year, prof. Friedrich Förster and dr. Tsviya Zeev-Ben-Mordehai, two experts in cryo-electron microscopy, will join our center. These new additions to the Bijvoet Center will further strengthen our scientific position and the first collaborative publications are already emerging!

Indeed, the level of coherence and collaboration within the Bijvoet Center is further increasing. A nice recent example of the power of scientific collaboration within our center refers to the study on the role of Axin mutants on Wnt signalling in cancer development published in Nature Structural and Molecular Biology, by the groups of Stefan Rüdiger, Albert Heck, Rolf Boelens and Madelon Maurice (Anvarian Z., Nat Struct Mol Biol. 2016 23:324-32). These findings hold significant potential for development of future cancer treatments.

Several members of the Bijvoet Center also received prestigious awards and grants in the past two years. In 2014, the Royal Netherlands Academy of Arts and Sciences awarded Celia Berkers with the Heineken Young Scientists Award for Biochemistry and Biophysics for her research into the workings of the proteasome, a structure that breaks down proteins in biological cells. In addition, Marc Baldus received the Günther Laukien Prize that recognizes cutting-edge experimental research in NMR in 2014. In 2015, Antoinette Killian was awarded the prestigious EBSA/Avanti prize for outstanding contributions to the understanding of lipid biophysics. In August 2015, the American Chemical Society honoured Albert Heck with the 'ACS Frank H. Field and Joe L. Franklin Award for Outstanding Achievements in Mass Spectrometry', and presented the 'Arthur C. Cope Scholar Award' to Geert-Jan Boons. Early 2016, Casper Hoogenraad was awarded the 2016 IBRO/Kemali International Prize for Research in the Field of Basic and Clinical Neuroscience.

Staff members of the Bijvoet Center were again very successful in obtaining competitive research grants, some of which we would like to mention here. In 2015, two TOP-PUNT grants of NWO Chemical Sciences were awarded to Marc Baldus together with Alexandre Bonvin and to Albert Heck together with Geert-Jan Boons. ZonMW granted TOP projects to Ineke Braakman and to a team of Casper Hoogenraad and Stefan Rüdiger. NWO Veni grants were to Rob de Vries of the Medicinal Chemistry group and Li Xue of the NMR spectroscopy group. Also from the NMR group, Markus Weingarth received in 2015 a Vidi grant. An NWO Vici grant was awarded to Madelon Maurice and an ERC Starting Grant was awarded to Bert Janssen. The European Union awarded a large grant from its Horizon 2020 programme to a project coordinated by Rolf Boelens, called iNEXT, to provide access to structural biology technologies to other researchers in Europe. And there were many, many more grants awarded to the Bijvoet Center in this period, as described in this report.

With a strong recent past, let's work on making the coming 2 years another milestone in the history of our center!

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Scientific Director

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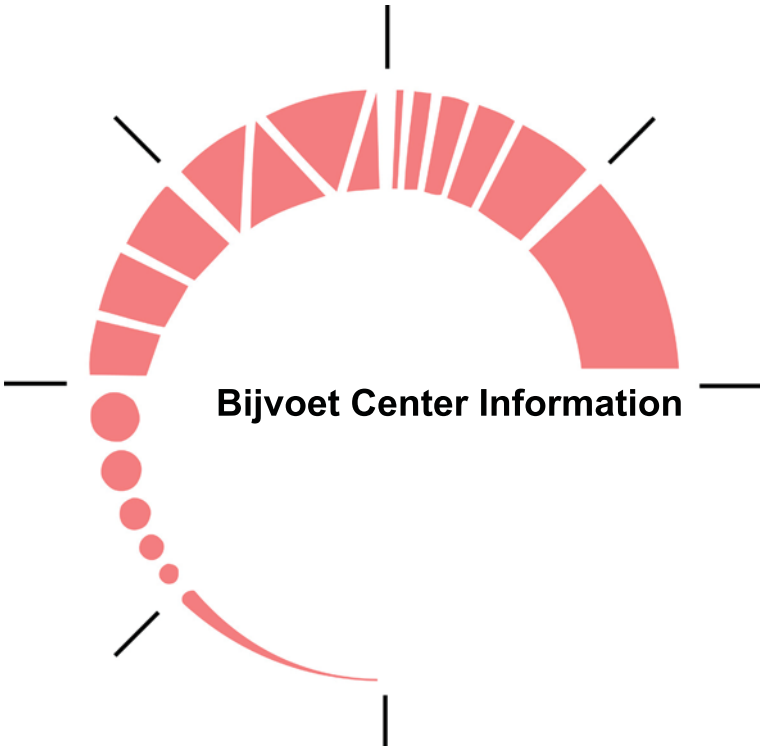
Colofon

Design by Reinout Raijmakers, photography by Ivar Pel, Bas van Breukelen, Reinout Raijmakers, and others.


Bijvoet Center Information

Organization

The Bijvoet Center for Biomolecular Research comprises several research groups, most of which are part of the Sciency Faculty of Utrecht University, (UU) and some are part of the University Medical Center Utrecht (UMCU).

Biomolecular Mass Spectrometry & Proteomics

The Biomolecular Mass Spectrometry and Proteomics groups develops mass spectrometry based enabling technologies for the structural and functional analysis and characterization of proteins and proteomes and applies them to significant research questions, to obtain biological relevant information. The group consists of about 50 people and is headed by Albert Heck and is embedded in both the Departments of Chemistry and Pharmaceutical Sciences. The group further consists of the principal investiagors Maarten Altelaar, Richard Scheltema (both Pharmaceutical Sciences), Celia Berkers and Simone Lemeer (both Chemistry), as well as dedicated educational staff.

Crystal and Structural Chemistry

The Crystal and Structural Chemistry group performs research into the three-dimensional structures of molecules, which define the molecular interactions and reactions that underlie complex chemical and biological processes. The main research lines are in the field of protein crystallography, chemical crystallography and the development of crystallographic methods. The Crystal and Structural Chemistry group consists of five principle investigators: Piet Gros, Eric Huizinga, Loes Kroon-Batenburg, Bert Janssen and Martin Lutz, who also is head of National Single Crystal Facility.

Medicinal Chemistry & Chemical Biology

The Medicinal Chemistry & Chemical Biology group focuses on design, chemo- or biosynthesis of compounds with biological or medicinal applications and interaction with their receptors to obtain new bio-active compounds and new approaches for the treatment of diseases. Especially modified peptides and peptidomimetics as well as carbohydrates are synthesized and studied. The Medicinal Chemistry and Chemical Biology group was expanded in 2015 when Geert-Jan Boons joined Utrecht University. In addition, the group includes Roland Pieters and a subgroup headed by Nathaniel Martin. The group is embedded in the Department of Pharmaceutical Sciences.

Membrane Biochemistry and Biophysics

The research in the Membrane Biochemistry and Biophysics group is focused on understanding the structure and function of biological membranes on a molecular level by studying the interactions between its main constituents: lipids and proteins. In addition they investigate how membranes are involved in the mode of action of drugs, toxins, antibiotics and amyloid forming proteins. Principal investigators in this group are Antoinette Killian, Toon de Kroon and Eefjan Breukink.

Cellular Protein Chemistry

The research in Cellular Protein Chemistry aims to characterize in molecular detail mechanisms of protein folding and chaperone action in mammalian cells, organelle biogenesis and maintenance, and chaperone-substrate interactions. Permanent scientific staff consists of Ineke Braakman and Stefan Rüdiger as also includes Bertrand Kleizen. who is also the coordinator of the MCLS master programme.

NMR Spectroscopy

The research of the NMR group aims at gaining atomic-level insight into biological processes. Solution and solid-state NMR spectroscopy are combined with molecular biology and computational structural biology methods to study processes involved in gene regulation, DNA repair, cellular signaling, biogenesis and membrane protein complexes. The group is headed by Marc Baldus and further includes Rolf Boelens, Alexandre Bonvin, Gert Folkers and Markus Weingarth as principal investigators.

Cryo-electron microscopy

The Cryo-EM research group in the Bijvoet Center was established early 2013 and focusses on studying biological systems on different scales – from 'cells to atoms' – with novel imaging and preparation methods. The aim is to develop and improve a workflow that integrates/combines different methodologies for in situ structural biology. Until early 2015, the group was headed by Jürgen Plitzko, who left Utrecht University for a position at the Max Planck Institute in Martinsried. In June 2016, Friedrich Förster will start as the new head of the Cryo-EM group. The group further includes principal investigators Wally Müller and Willie Geerts.

Timmers group

The research of Marc Timmers focuses on the interplay between chromatin modifications and basal transcription factors like TFIID. Until December 2015, he was part of the Molecular Cancer Research group of the University Medical Center Utrecht, but he has moved to the new Center for Regenerative Medicine at the Utrecht Science Park the Uithof, a joined initiative between the Hubrecht Institute, the UMCU and Utrecht University.

Associate Members

Several other excellent researchers at UU and the UMCU are Associate Members of the Bijvoet Center. They collaborate with the groups in the Bijvoet Center and strengthen the link between the Bijvoet Center and the UMCU. The current Associate Members of the center are Madelon Maurice (UMCU, Cell Biology), Holger Rehmann (UMCU, Molecular Cancer Research), Anna Akhmanov (UU, Cell Biology) and Casper Hoogenraad (UU, Cell Biology).

Infrastructures

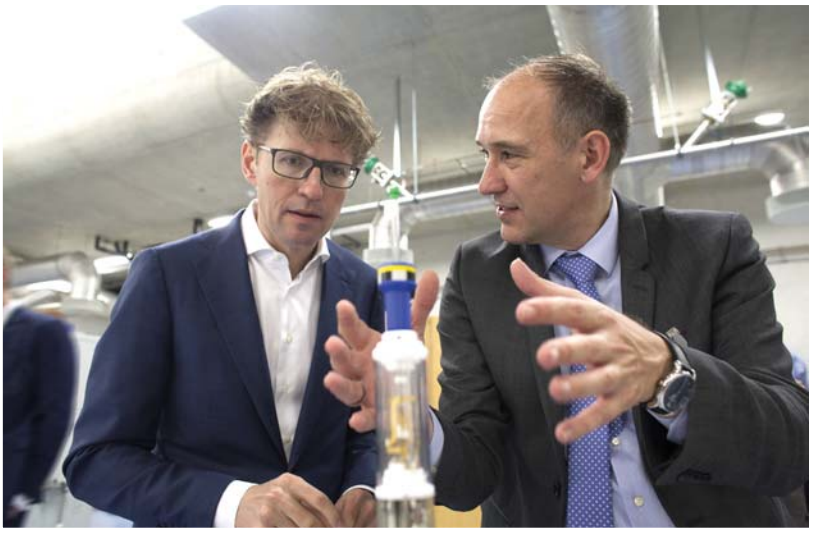
The Bijvoet Center is also home to several infrastructures, supporting both the research in the Center as well as that of many researchers throughout the Netherlands and Europe.

Crystallography

The National Single Crystal X-ray Facility at the Bijvoet Center offers crystal structure determinations to synthetic chemists at universities, institutes and companies in the Netherlands. With experienced staff, state-of-the-art equipment and advanced software it can deal with a large variation of chemical compounds and in case of academic collaborations the facility contributes also to the preparation of the scientific publications. The facility houses two modern diffractometers, a Bruker Kappa Apex II which is suitable for measurements at low temperature and which is routinely used for all kinds of organic and organometallic crystals and a Bruker Proteum that is especially suited for protein crystallography and for small and weakly diffracting crystals in chemical crystallography.

NMR Spectroscopy

The Utrecht Facility for High-Resolution NMR is hosted by the NMR group of the Bijvoet Center. Funded by the Council for Chemical Research of the Netherlands Organization for Scientific Research (NWO-CW), the Utrecht NMR Facility makes its advanced NMR infrastructure and specialist expertise available to Dutch guest researchers. Access to NMR instrumentation for researchers from EU countries and Associated States is provided in the context of the Horizon 2020 project iNEXT, which is coordinated by Rolf Boelens.



Dutch state secretary Sander Dekker (left) and Marc Baldus (right) at the official opening of the uNMR-NL facility in November 2015.

At the NMR group, Marc Baldus is the coordinator of the uNMR-NL NWO Roadmap project, which is aimed at eventually installing a 1.2 GHz NMR spectrometer for the Netherlands. In November 2015, a new 950 MHz spectrometer was installed for the uNMR-NL project and on November 5, 2015, Dutch state secretary Sander Dekker officially opened this new national NMR facility at the Bijvoet Center.

Mass Spectrometry & Proteomics

The Biomolecular Mass Spectrometry and Proteomics Group forms the core of the Netherlands Proteomics Centre (NPC), a strategic collaboration of proteomics research groups throughout the Netherlands. At Utrecht University, access is provided for large scale proteomics experiments as well as native MS for structural analysis of proteins and protein complexes. Access is provided to 20 state-of-the-art high resolution mass spectrometers. Access to the facilities for scientists from the European Union was provided in the context of the FP7 funded project PRIME-XS until early 2015. Since 2014, the NPC provides access to Dutch and international researchers through the NWO Roadmap project *Proteins@Work*.

Electron Microscopy

Early 2016, the Bijvoet Center established, together with the Debye Institute for Nanomaterials and the Faculty of Geosciences, a new facility for electron microscopy, called the EM Square at Utrecht University.

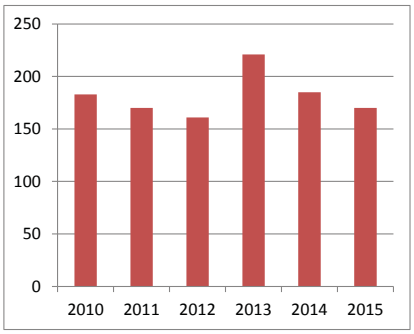
The facility is devoted to the development and application of electron microscopy methodologies for life sciences, geosciences, and material sciences research, covering the entire range of specimen preparation, electron microscopy data collection and analysis, and 3D reconstruction techniques. The infrastructure includes several advanced Transmission Electron Microscopes (TEM) and Scanning Electron Microscopes (SEM), including state-of-the-art equipment for cryo-electron tomography and single particle analysis.

Instruct

Since May 2013, the infrastructure of the Bijvoet Center, together with the electron microscopy facility NeCEN in Leiden and the Protein Facility of the Netherlands Cancer Institute in Amsterdam is a full Instruct Center within the ESFRI project Instruct.

Scientific Output

The scientists in the Bijvoet Center published 185 papers in 2014 and 170 papers in 2015, which is well in line with the output over the past years:



Total number of publications from the Bijvoet Center over the last years.

Output per group

The distribution of these publications over the various research groups is shown in the figure to the right. Please note that publications between multiple research groups in the center are counted towards the total of both groups (*BMS: Biomolecular Mass Spectrometry and Proteomics; CSC: Chemical and Structural Crystallography; CPC: Cellular Protein Chemistry; MC: Medicinal Chemistry & Chemical Biology; NMR: NMR spectroscopy; MBB: Membrane Biochemistry & Biophysics; MCR: Timmers Group; BOC: former Bio-Organic Chemistry; Associated: Associate Members*).

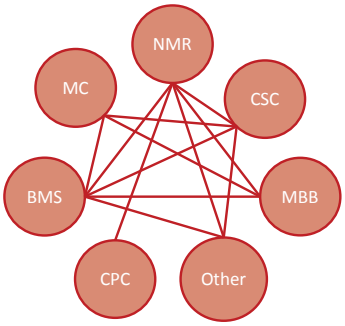
Impact of publications

The average impact factor of the journals in which the groups published was 7.6 over 2014 and 7.2 over 2015, based on the Thompson JCR 2013 index.

In total, 70 articles were published in journals with an impact greater than 10 of which 15 in journals with an impact over 20 (see box below).

Collaboration

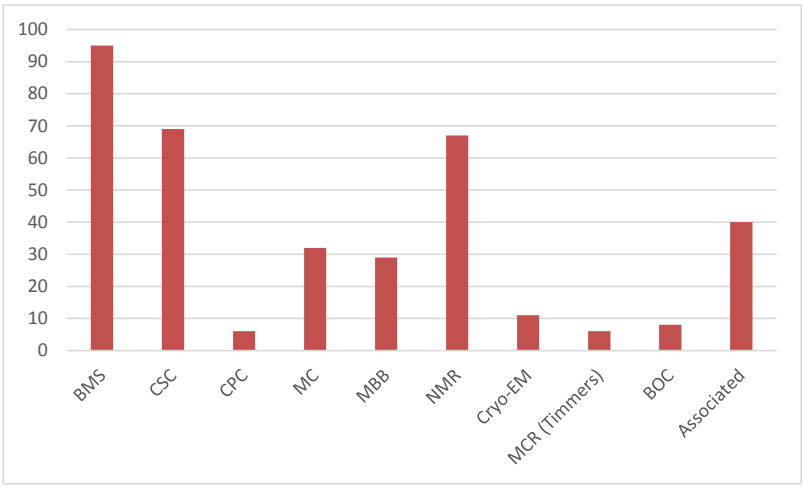
There have been 21 joint publications between groups in the Bijvoet Center, and these have involved almost all groups in the center in varying combinations, as is shown in the graph on the top right.



Lines between research groups indicates that they have jointly published research articles in 2014 and/or 2015.

Journals

The journals in which most often was published were Angewandte Chemie (14x), Molecular and Cellular Proteomics (12x), Journal of Biological Chemistry (10x) and Organometallics (10x).



Top publications '14-'15 (impact>20)

Akhmanova, A; Steinmetz, MO (2015) "Control of microtubule organization and dynamics: two ends in the limelight" **Nat. Rev. Mol. Cell Biol.** 16:711-726

Benoit, RM; Frey, D; Hilbert, M; Kevenaar, JT; Wieser, MM; Stirnimann, CU; McMillan, D; Ceska, T; Lebon, F; Jaussi, R; Steinmetz, MO; Schertler, GFX; Hoogenraad, CC; Capitani, G; Kammerer, RA (2014) "Structural basis for recognition of synaptic vesicle protein 2C by botulinum neurotoxin A" **Nature** 505:108

Consonni, SV; Maurice, MM; Bos, JL (2014) "DEP domains: structurally similar but functionally different" **Nat. Rev. Mol. Cell Biol**. 15:357-362

D'Astolfo, DS; Pagliero, RJ; Pras, A; Karthaus, WR; Clevers, H; Prasad, V; Lebbink, RJ; Rehmann, H; Geijsen, N (2015) "Efficient Intracellular Delivery of Native Proteins" **Cell** 161:674-690

Diebolder, CA; Beurskens, FJ; de Jong, RN; Koning, RI; Strumane, K; Lindorfer, MA; Voorhorst, M; Ugurlar, D; Rosati, S; Heck, AJR; van de Winkel, JGJ; Wilson, IA; Koster, AJ; Taylor, RP; Saphire, EO; Burton, DR; Schuurman, J; Gros, P; Parren, PWHI (2014) "Complement Is Activated by IgG Hexamers Assembled at the Cell Surface" **Science** 343:1260-3

Dreijerink, KMA; Conemans, EB; Cornelissen, C; van Blokland, MT; ... ; Havekes, B; Fliers, E; Hermus, AR; Vriens, MR; Timmers, HT; van der Wall, E; Brown, M; van der Luijt, RB; van Diest, PJ; Goudet, P; Burgess, JR; Valk, GD (2014) "Breast-Cancer Predisposition in Multiple Endocrine Neoplasia Type 1" **N. Engl. J. Med.** 371:583-584

Holthuis, JCM; Menon, AK (2014) "Lipid landscapes and pipelines in membrane homeostasis" **Nature** 510:48-57

Hussein, SMI; Puri, MC; Tonge, PD; Benevento, M; Corso, AJ; Clancy, JL; Mosbergen, R; Li, M; Lee, DS; Cloonan, N; Wood, DLA; Munoz, J; Middleton, R; Korn, O; Patel, HR; White, CA; Shin, JY; Gauthier, ME; Le Cao, KA; Kim, JI; Mar, JC; Shakiba, N; Ritchie, W; Rasko, JEJ; Grimmond, SM; Zandstra, PW; Wells, CA; Preiss, T; Seo, JS; Heck, AJR; Rogers, IM; Nagy, A (2014) "Genome-wide characterization of the routes to pluripotency" **Nature** 516:198

Kaplan, M; Cukkemane, A; van Zundert, GCP; Narasimhan, S; Daniels, M; Mance, D; Waksman, G; Bonvin, AMJJ; Fronzes, R; Folkers, GE; Baldus, M (2015) "Probing a cell-embedded megadalton protein complex by DNP-supported solid-state NMR" **Nat. Methods** 12:649

Karagoz, GE; Duarte, AMS; Akoury, E; Ippel, H; Biernat, J; Luengo, TM; Radli, M; Didenko, T; Nordhues, BA; Veprintsev, DB; Dickey, CA; Mandelkow, E; Zweckstetter, M; Boelens, R; Madl, T; Rudiger, SGD (2014) "Hsp90-Tau Complex Reveals Molecular Basis for Specificity in Chaperone Action" **Cell** 156:963-974

Kolinko, I; Lohsse, A; Borg, S; Raschdorf, O; Jogler, C; Tu, Q; Posfai, M; Tompa, E; Plitzko, JM; Brachmann, A; Wanner, G; Muller, R; Zhang, YM; Schuller, D (2014) "Biosynthesis of magnetic nanostructures in a foreign organism by transfer of bacterial magnetosome gene clusters" **Nat. Nanotechnol.** 9:193-197

Koster, MJE; Snel, B; Timmers, HTM (2015) "Genesis of Chromatin and Transcription Dynamics in the Origin of Species" **Cell** 161:724-736

Liu, F; Rijkers, DTS; Post, H; Heck, AJR (2015) "Proteome-wide profiling of protein assemblies by cross-linking mass spectrometry" **Nat. Methods** 12:1179

Pedersen, LB; Akhmanova, A (2014) "Kif7 keeps cilia tips in shape" **Nat. Cell Biol.** 16:623-625

van Bergeijk, P; Adrian, M; Hoogenraad, CC; Kapitein, LC (2015) "Optogenetic control of organelle transport and positioning" **Nature** 518:111

The scientists in the Bijvoet Center have been very successful in acquiring funding in 2014 and 2015. In both years, the total amount of funding acquired for research from funding agencies and companies was around 10 M€. Below are some of the most notable grants and awards received by the scientists of the Bijvoet Center in 2014 and 2015.

NWO

In 2015, two TOP-PUNT grants (2 M€ per grant) of NWO Chemical Sciences were awarded to the Bijvoet Center. The research teams that received the grants were Marc Baldus together with Alexandre Bonvin, both of the NMR spectroscopy group (*"Caught in the act: a combined magnetic resonance – modelling approach to capture cellular machines at work"*) and Albert Heck of the Biomolecular Mass Spectrometry and Proteomics group together with Geert-Jan Boons of the Medicinal Chemistry group (*"Combining chemical synthesis and analysis to reveal the biology regulated by protein glycosylation"*).

ZonMW granted TOP projects (675 k€ each) to Ineke Braakman of the Cellular Protein Chemistry group, (*"Genezing van Taaislijmziekte dichtbij"*), in collaboration with prof. dr. van der Ent (UMCU), dr. Beekman (UMCU) and dr. de Jonge (ErasmusMC) and to associate member Casper Hoogenraad and Stefan Rüdiger of the Cellular Protein Chemistry group, (*"Chaperoning axonal transport in neurodegenerative disease"*), in collaboration with dr. Toonen (VU Amsterdam).

Piet Gros was awarded an NCI Technology Area project from NWO Chemical Sciences (1.58M€ total, *"Targeting membrane proteins"*) together with dr. Parren (Genmab) dr. Hemrika (U-Protein Express), dr. van Bergen en Henegouwen (UU, Biology) and dr. van Diest (UMCU).

Personal Grants

NWO Veni grants (250 k€ each) were awarded in 2014 to Rob de Vries of the Medicinal Chemistry group (*"Inhibiting the highly diverse receptor binding pocket of Influenza A Virus using its conserved ligand"*) and Li Xue of the NMR spectroscopy group (*"Shrinking protein-RNA conformational space with artificial intelligence"*).

Markus Weingarth of the NMR Spectroscopy group received an NWO Vidi grant (800 k€) in 2015 for his project *"In-cell monitoring of membrane proteins at atomic-resolution"*. An NWO Vici grant (1.5 M€) was awarded to associate member Madelon Maurice in 2015 (*"Controlling the controller: Regulation of signals that guide stem and cancer cell growth and differentiation"*).

The European Research Council awarded an ERC Starting Grant (1.5 M€) to Bert Janssen of the Crystal and Structural Chemistry group for the project *"Molecular Adhesion and Interactions in the Nervous system"*.

Fellowships

Dimphna Meijer, working in the Crystal and Structural Chemistry group, received an EMBO long term fellowship. Anna Vangone and Irina Moreira, both working in the NMR spectroscopy group, received Marie Curie Individual Fellowships from the Horizon 2020 programme.

Horizon 2020

The European Union awarded 10 M€ from its Horizon 2020 programme to the project iNEXT, coordinated by Rolf Boelens of the NMR spectroscopy group, which aims to provide integrated access to structural biology technologies such as NMR, electron microscopy and X-ray technologies.

Alexandre Bonvin was awarded a total of 900 k€ through participation in four Horizon 2020 funded eInfrastructure projects.

Albert Heck, together with colleagues in Germany and Denmark, was awarded 3.7 M€ for an EU FET OPEN project to make large-scale protein analysis clinically applicable.

Other

Roland Pieters of the Medicinal Chemistry group and Richard Scheltema of the Biomolecular Mass Spectrometry and Proteomics group were awarded Seed Grants from the Utrecht Institute for Pharmaceutical Sciences (UIPS)

Ineke Braakman received funding from the Netherlands Cystic Fibrosis Foundation, the foundation Muco & Friends and the Topsector Life Science & Health for her research on Cystic Fibrosis.

The Dutch Kidney foundation awarded a grant to a consortium including Piet Gros to study the role of complement in kidney disease.

Awards

In August 2015, the American Chemical Society honoured Albert Heck with the 'ACS Frank H. Field and Joe L. Franklin Award for Outstanding Achievements in Mass Spectrometry', and presented the 'Arthur C. Cope Scholar Award' to Geert-Jan Boons. Heck received the award for his development of new methods and techniques to identify and study the structure and function of proteins and protein complexes. Boons received the award for his contribution to our understanding of the structure and function of complex sugars in human cells.

Antoinette Killian of the Membrane Biochemistry and Biophysics group was awarded the prestigious EBSA/Avanti prize in 2015. This biennial award, established by Avanti Polar Lipids is given for outstanding contributions to the understanding of lipid biophysics.

Casper Hoogenraad was awarded the 2016 IBRO/Kemali International Prize for Research in the Field of Basic and Clinical Neuroscience. Hoogenraad received the award for his "outstanding work on cytoskeleton dynamics and intracellular transport in neural development and synaptic plasticity".

Bijvoet School

The Bijvoet School provides education in the *Molecular and Cellular Life Sciences* master student programme and the PhD student programme *Molecular Life Science*, part of the *Graduate School of Life Sciences* of Utrecht University

Seminars

The Bijvoet School organizes regular seminars where external scientists present their research to the students, postdocs and staff of the center. These people were invited for Bijvoet Seminars in 2014 and 2015:

Daniel Southworth (11 Mar, 2014)
Henry van den Bedem (2 Jul, 2014)
Remco Sprangers (22 Sep, 2014)
Eduardo Perozo (28 Oct, 2014)
Yvonne Jones (12 Nov, 2014)
Kevin H. Mayo (10 Dec, 2014)
Nicky Packer (13 Mar, 2015)
Alan Mark (31 Mar, 2015)
Juliane Liepe (19 May, 2015)
Friedrich Förster (19 May, 2015)
Niels de Jonge (20 May, 2015)
Chris Abell (28 Aug, 2015)
Katja Petzold (10 Sep, 2015)
John Briggs (1 Oct, 2015)
Sandro Keller (19 Nov, 2015)

Tutorial Symposium

The annual Bijvoet tutorial symposia are dedicated to many aspects of the structure, function and interaction of biomolecules. In 2014 it was a two day meeting and in 2015 a one day meeting. The meetings included the following keynote speakers:

2014
Susan Lea (University of Oxford)
Phil Selenko (FMP Berlin)
Mitsu Ikura (University of Toronto)
Dirk Slotboom (University of Groningen)
Anna Akhmanova (Utrecht University)

2015
Ophry Pines (Hebrew University of Jerusalem)
Maya Topf (University of London)
Casper Hoogenraad (Utrecht University)

Each year a PhD student is selected as PhD student of the year by the Scientific Advisory Board. This student is given the opportunity to present their research at the Bijvoet Symposium. These students were given the honour in the past two years:

2014
Weng Chuan Peng (Crystal and Structural Chemistry)

2015
Joost Snijder (Biomolecular Mass Specrtometry and Proteomics)

Bachelor student programme

In September 2014, a new "Molecular Life Science" bachelor track started within the Chemistry curriculum, which is a joint effort of the people in the Bijvoet Center with colleagues from the Departments Biology and Pharmaceutical Sciences. The programme has attracted almost 50 students per year and is now in its second year

Master student programme

The master student programme *Molecular and Cellular Life Sciences*, in which the Bijvoet School participates, focuses on understanding molecular-level cellular function and rests at the crossroads of chemical, biological, physical and computational science. The programme attracts around 50 students per year.

PhD student programme

The *Molecular Life Science* programme, provided by the Bijvoet School, part of the Bijvoet Center for Biomolecular Research, aims to provide PhD students with a solid and broad knowledge of structural biochemistry. The PhD students are given an introductory afternoon to get to know the center. They are expected to attend the Bijvoet Tutorial Symposium and the Bijvoet Seminars, present their research to each other at regular "PhD student evenings" and are offered a variety of courses.

Courses

The programme provides training opportunities for PhD students interested in all areas of structural biology available in the Bijvoet Center: X-ray diffraction, mass spectrometry, proteomics, solid-state and liquid-state NMR, protein folding, membrane enzymology and lipidomics.
The following courses are all organized on a regular basis and are open to all PhD students in the Graduate School of Life Sciences.

- Advanced NMR Spectroscopy
- Biomolecular Mass Spectrometry
- Advanced Protein Crystallography

In addition to these courses, many other courses organized by different programmes in the Graduate School of Life Sciences are open to the PhD students in the programme.

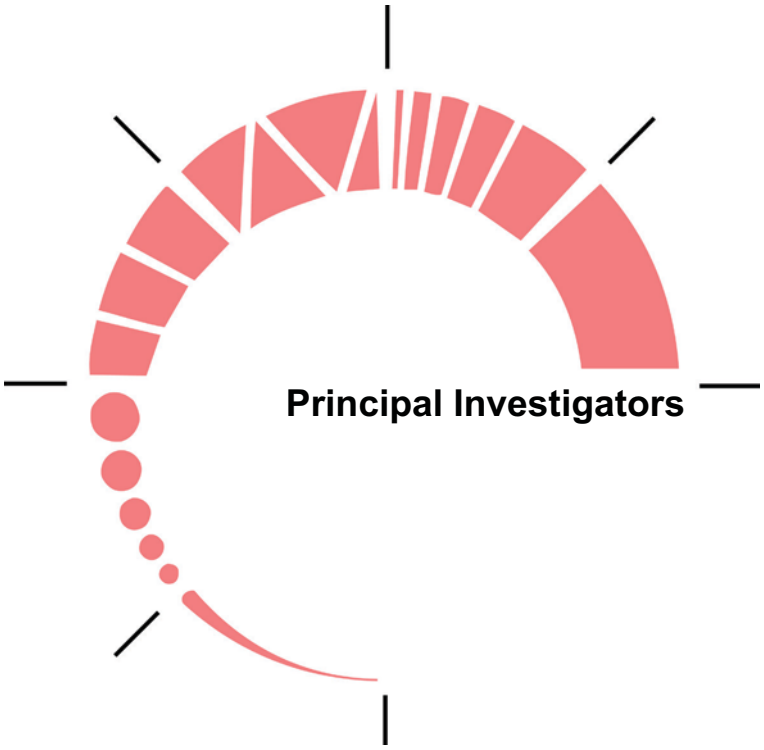
PhD students

The groups of the Bijvoet Center currently have around 100 active PhD students, with 61 of them enrolled in the *Molecular Life Science* programme of the Bijvoet School.

After graduation, around 75% of the PhD students of the Bijvoet School continue their career in academia and approximately half of all graduated PhD students find their next job outside of the Netherlands, with most of them going to either other European countries or to Northen America.

Summerschool

Since 2010, the Bijvoet School also organizes, together with *The Utrecht Summer School*, a summerschool called "Exploring Nature's Molecular Machines" on the chemical principles underlying protein structure and function and how proteins form an assembly of "molecular machines". The target audience of the summerschool are advanced bachelor students or beginning master students in the early stage of their study with a background in chemistry, molecular biology or biophysics.


Principal Investigators

Anna Akhmanova

Associate Member

Anna Akhmanova studied biochemistry and molecular biology at the Moscow State University. She received her PhD in 1997 at the University of Nijmegen. She worked as a post-doc at the Department of Microbiology and Evolutionary Biology at the University of Nijmegen and at the Department of Cell Biology at the Erasmus Medical Center in Rotterdam. In 2001, she has started her own research group at the Department of Cell Biology at the Erasmus Medical Center. Since 2011, Anna Akhmanova is professor of Cell Biology at Utrecht University. Anna Akhmanova is a recipient of the ALW Vernieuwingsimpuls VIDI (2001) and VICI awards (2007), and an ERC Synergy grant (2013). She is a member of the European Molecular Biology Organization (EMBO), the Royal Netherlands Academy of Arts and Sciences (KNAW) and the Chair of the board of the Netherlands Society for Microscopy.

Contact

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Selected publications

Akhmanova, A. et al. Control of microtubule organization and dynamics: two ends in the limelight. Nat Rev Mol Cell Biol. 16:711-26 (2015)

Doodhi, H. et al. Mechanical and geometrical constraints control kinesin-based microtubule guidance. Curr Biol. 24:322-8 (2014)

Jiang, K. et al. Microtubule minus-end stabilization by polymerization-driven CAMSAP deposition. Dev Cell. 28:295-309 (2014)

The Akhmanova lab studies cytoskeletal organization and trafficking processes, which contribute to cell polarization, differentiation, vertebrate development and human disease. The main focus of the work in Akhmanova lab is the microtubule cytoskeleton. Research in the group relies on combining high-resolution live cell imaging and quantitative analysis of cytoskeletal dynamics, measurement of protein dynamics using advanced microscopic assays, in vitro reconstitution of dynamic cytoskeleton-based processes and different methods of identification of protein-protein interactions. In collaboration with mathematicians, the lab is working on development of automated analysis and modeling of cytoskeletal dynamics and vesicle transport.

Microtubules are cytoskeletal polymers with two structurally and functionally distinct ends, the plus- and the minus-end. A wealth of information exists on microtubule plus ends and the proteins which bind to them, such as EB1 and its partners. In contrast, much less is known about the minus ends. Aklhmanova lab currently focuses on the mechanisms underlying the regulation of microtubule minus-ends by the CAMSAP/Nezha/Patronin protein family. In a recent study, the group showed that CAMSAP2 is required for the proper organization and stabilization of interphase microtubules and directional cell migration. By combining live-cell imaging and in vitro reconstitution of microtubule assembly from purified components with laser microsurgery, it was demonstrated that CAMSAPs regulate microtubule minus-end growth and are specifically deposited on the lattice formed by microtubule minus-end polymerization. This process leads to the formation of CAMSAP-decorated microtubule stretches, which are stabilized from both ends and serve as sites of non-centrosomal microtubule outgrowth. The length of the stretches is regulated by the microtubule-severing protein katanin, which interacts with CAMSAPs. These data indicate that microtubule minus-end assembly drives the stabilization of non-centrosomal microtubules and that katanin regulates this process.

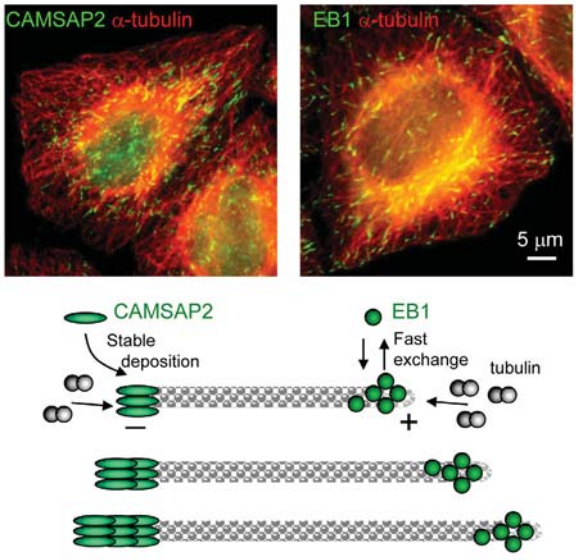

Figure: Illustration of the distribution of microtubule minus ends labeled with CAMSAP2 and microtubule plus ends labeled with EB1 in Hela cells (the two microtubule end markers are shown in green, microtubules in red). A scheme illustrating the accumulation of CAMSAP2 and EB1 at the two dynamic microtubule ends is shown below.

Maarten Altelaar

Research in the Altelaar group focuses on the development and implementation of innovative proteomics methods for the more efficient and detailed characterization of proteins, protein complexes and their post-translational modifications (PTMs) in relation to their biological function in health and disease. Main emphasis is on the improvement of sensitivity, specificity and throughput of proteomics technology, focused on cellular signaling in cancer proteomics, drug resistance, immunotherapy and stem cell differentiation. As such my lab has setup label free phosphopeptide quantification in combination with targeted mass spectrometry (SRM) to monitor pathway specific phosphorylation dynamics. More recently, my group has implemented multi-omics approaches to understand system-wide biological responses to perturbations in the light of drug sensitivity and resistance. My research group contains a mix of expertise in mass spectrometry, biology, chemistry and pharmacy. We tackle larger proteomics projects as a team and have strong collaborations with specialists in the field of molecular oncology at the Dutch Cancer Institute and stem cell differentiation at the Utrecht University and Medical Centre.

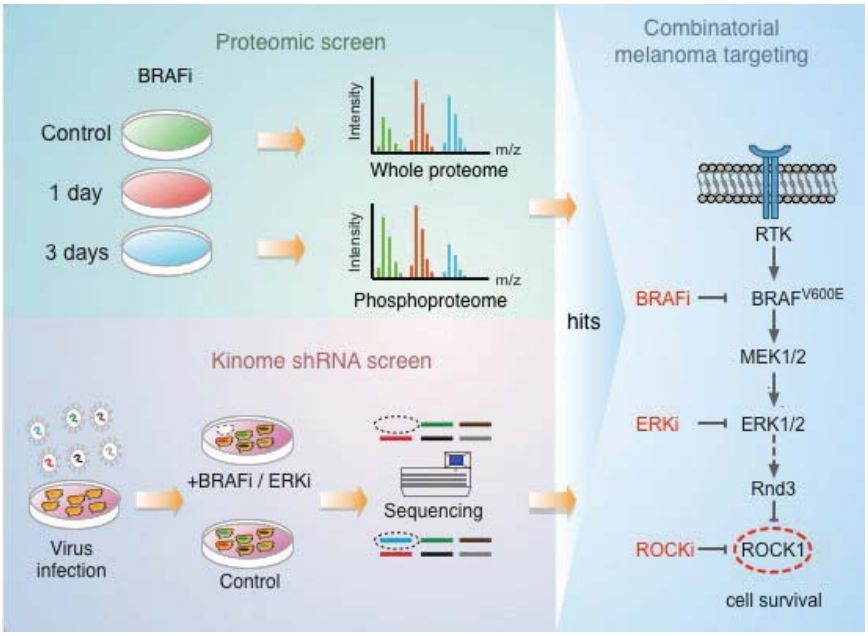

Figure: Integrated proteomics and genomics analysis revealed ROCK1 as potential co-target in BRAF mutant melanoma.

Maarten Altelaar studied analytical chemistry at the Vrije Universiteit (VU) in Amsterdam, where he received his Masters degree in July 2002. In May 2002 he started a PhD under at the FOM-Institute for Atomic and Molecular Physics (AMOLF). Topic of his research was the development of methodology for imaging mass spectrometry, mapping biomolecular distributions directly from single cell and tissue surfaces. In March 2007 he received his PhD after which he joined the Biomolecular Mass Spectrometry and Proteomics group at the University of Utrecht. Here the focus of his research switched to more proteomics based techniques, with the emphasis on quantitation, electron transfer dissociation (ETD) and cancer and neuroproteomics. Maarten is currently appointed as associate professor at the Utrecht University and in 2013 received an NWO VIDI award on a proposal named Network Medicine; Quantifying Proteome Wide Crosstalk, aimed at the development of proteomics methodology for the quantitative analysis of protein-protein interactions, PTMs and protein signaling networks in the face of their interconnectivity, in BRAF mutant melanoma drug resistance.

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Selected publications

Schmidlin, T. et al. Diet-Induced europeptide Expression: Feasibility of Quantifying Extended and Highly Charged Endogenous Peptide Sequences by Selected Reaction Monitoring. Analytical Chemistry 87:9966-9973 (2015)

Raaijmakers, L.M. et al. PhosphoPath: Visualization of phosphosite-centric dynamics in temporal molecular networks. J. Prot. Res. 14: 4332-4341 (2015)

Smit, M.A. et al. ROCK1 is a potential combinatorial drug target for BRAF mutant melanoma. Mol. Sys. Biol. 10:772 (2014)

Marc Baldus

Marc Baldus obtained his Ph.D. from ETH Zürich in 1996 and worked as postdoctoral research associate at MIT, Cambridge/USA. (1997-1999). After a university Lecturership at the Leiden Institute of Chemistry, he became group leader at the Max-Planck-Institute for Biophysical Chemistry in Göttingen in 2000. He returned to the Netherlands to become full Professor of Structural Biology (2008) at Utrecht University and has been heading its NMR section since 2010. He received the Founders Medal of the International Council on Magnetic Resonance in Biological Systems (ICMRBS) in 2006, the EBSA prize of the Association of the European Biophysical Societies (2007) and the Günther Laukien Prize (2014). He acquired NWO VICI and NWO-groot (2010) funding as well as an NWO Top-punt grant (2015, with A. Bonvin) to conduct NMR-based research at the interface of structural and cellular biology. Since 2012, he coordinates the NWO Roadmap project uNMR-NL to set up a 1.2 GHz NMR facility for the Netherlands. Phase 1 of this project, i.e., installation of a 950 MHz NMR in Utrecht, was completed in November 2015. Since April 2016, he is scientific director of the Bijvoet Center.

Contact

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Selected publications

Kaplan, M. et al. Probing a cell-embedded Megadalton protein complex by DNP-supported solid-state NMR, Nature Methods 12:649-652 (2015)

Jantschke, A. et al. Insight into the Supramolecular Architecture of Intact Diatom Biosilica from DNP-Supported Solid-State NMR Spectroscopy, Angew. Chem. Int. Ed. 54:15069-15073 (2015)

Weingarth, M. et al. Quantitative analysis of the water occupancy around the selectivity filter of a K+ channel in different gating modes, J. Am. Chem. Soc. 136:2000-2007 (2014)

Bacteria have evolved a variety of highly specialized macromolecular nanomachines that secrete a wide range of substrates, including small molecules, proteins and DNA. An example is the type 4 secretion system (T4SS) that is responsible for the transfer of effector proteins and nucleic acids between bacteria and host cells and plays a vital role in bacterial conjugation thereby assisting the spread of antibiotic resistance. T4SS spans the entire bacterial cell envelope including the inner and outer membrane (Figure) and consists of 12 proteins, with three of these proteins (namely VirB7, VirB9 and VirB10) forming the so-called core complex (T4SScc). Thus far, high-resolution structural data are available for only half of the complex, including a low-resolution EM map. However, these results obtained on purified T4SScc cannot explain how this complex is located in its physiological environment and how substrates interact with this machine in a native setting.

For this reason, we resorted to investigate T4SScc in a native bacterial cell envelope by Dynamic Nuclear Polarization (DNP) enhanced solid-state NMR (ssNMR). We could successfully produce fully and specifically [13C, 15N] labeled T4SScc in E.coli lacking OmpA/F (thereby decreasing the NMR background signal) and isolate the cell envelope for in-situ DNP-enhanced ssNMR/DNP studies (Kaplan et al. 2015). In addition to T4SScc, other endogenous cellular compounds such as lipids and peptidoglycans were visible in our NMR spectra. Using our cellular ssNMR approach (see, e.g., also Renault et al, PNAS 2012), we could for the first time confirm that segments of the T4SScc seen in protein crystals retain their fold in the cellular envelope and we obtained new insight regarding the structure and dynamics of the hitherto elusive part of T4SScc, in particular its embedding in the inner bacterial membrane (Figure). Further NMR studies to refine structure and dynamics of the T4SScc in a native setting and in response to substrates are ongoing.

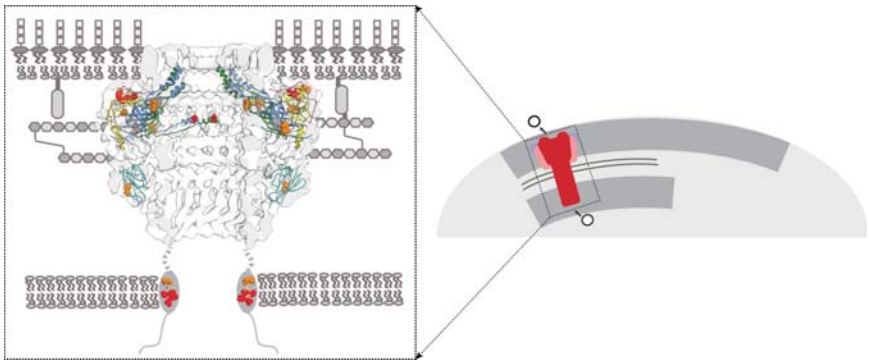

Figure: NMR zooms in on atomic scale on a large, cell-embedded protein complex (T4SScc) that bacteria use to secrete molecules. Orange and red balls show structural elements identified by NMR in a model that also includes microscopy and X-ray diffraction data previously obtained outside the cell environment.

Celia Berkers

The Berkers group combines Metabolomics – studying the small molecule metabolite profile of cells using Mass Spectrometry – with biochemical and chemical biology techniques. By comprehensively profiling metabolic changes in either drug resistant cells or in specific populations of immune cells, we aim to identify novel targets for therapeutic intervention. One of our research lines aims at studying T-cell metabolism. Immune responses depend on the balance between two types of T cells: conventional T cells (Tconv), that kill infected or tumor cells, and regulatory T cells (Treg), that inhibit the response of conventional T cells to self- and foreign antigens. Treg are critical to inhibit autoimmunity. Thus, patients with autoimmune diseases such as rheumatoid arthritis will likely benefit from Treg stimulation. On the other hand, Treg impede anti-tumor immunity and therefore, cancer patients will likely benefit from Treg inhibition. But because Treg and Tconv share many features - including co-stimulatory and cytokine receptors - compounds that selectively modulate different T-cell types are scarce. One aspect in which Treg may differ significantly from Tconv is in their metabolic features. Upon activation in lymphoid organs, T cells proliferate extremely fast and this places unique demands on their metabolism. But whereas proliferation of Tconv is depending on the metabolic master regulator mTORC1, Treg proliferation is not. In an Institute for Chemical Immunology project we aim, together with immunologist at the NKI and Sanquin, to map the metabolic differences between Treg and Tconv using a metabolomics approach. To this end, we use state-of-the-art LC/MS and combine steady-state metabolomics screens with metabolic flux studies using stable isotope-labeled nutrients. By studying not only in vitro expanded T cells but also freshly isolated primary human T-cell populations, we now start to see different metabolic signatures in Treg and Tconv, especially during T-cell activation. Further exploration of these differences may aid in the identification of novel targets that can be exploited to selectively modulate T-cell activity.

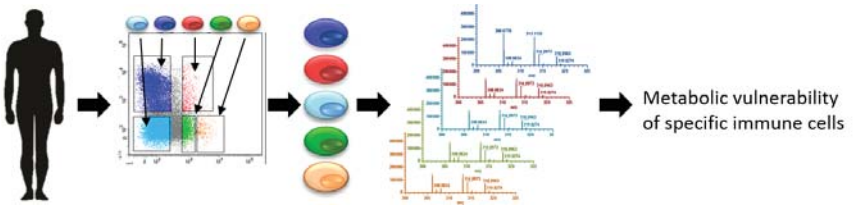

Figure: Autoimmune diseases and cancer are characterized by unbalanced levels of immune cells. By studying primary human T-cell populations using an LC/MS-based metabolomics approach we aim to identify metabolic vulnerabilities of specific T-cell populations that may be exploited for therapy.

Celia Berkers studied chemistry at Utrecht University, where she graduated cum laude in 2003. She started her PhD at the Harvard Medical School in Boston and continued her research at the Netherlands Cancer Institute in Amsterdam. Berkers received her PhD degree with honors in 2010 and was awarded the Antoni van Leeuwenhoek Prize 2010 for most promising young scientist. She then moved to the Beatson Institute for Cancer Research in Glasgow, where she did a post-doc in the laboratory of Prof. Karen Vousden, supported by a Rubicon fellowship from NWO. In 2013, Dr Berkers joined the Biomolecular Mass Spectrometry and Proteomics Department at Utrecht University as an independent group leader and was awarded a VENI grant from NWO-CW. In 2014, she was awarded the Heineken Young Scientists Award for Biochemistry and Biophysics by the Royal Netherlands Academy of Arts and Sciences.

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Selected publications

Berkers, C.R. et al., Definition of Proteasomal Peptide Splicing Rules for High-Efficiency Spliced Peptide Presentation by MHC Class I Molecules. J Immunol. 195, 4085-4095 (2015).

Berkers, C.R. et al. Peptide Splicing in the Proteasome Creates a Novel Type of Antigen with an Isopeptide Linkage. J Immunol. 195, 4075-4084 (2015).

D'Alessandro, A. et al. Metabolic effect of TAp63α: enhanced glycolysis and pentose phosphate pathway, resulting in increased antioxidant defense. Oncotarget 5, 7722-7733 (2014).

Rolf Boelens studied physical chemistry at the University of Groningen (Netherlands) and completed his Ph.D. in the Department of Biochemistry of the University of Amsterdam. For a postdoc he went to the NMR group of Robert Kaptein. Currently he is professor of biomolecular NMR spectroscopy and head of the Department of Chemistry. He is director of the European high-resolution NMR facility at Utrecht University and coordinates the European program iNEXT, a Research Infrastructure for NMR, EM and X-rays for Translational research. His research interests are biomolecular recognition, dynamics and function with on an emphasis on transcription and DNA repair involved in DNA transcription and DNA repair. This research is complemented by developing methods for biomolecular NMR. His research has resulted in over 340 publications.

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Selected publications

Faridounnia, F. et al. The Cerebro-oculo-facio-skeletal Syndrome Point Mutation F231L in the ERCC1 DNA Repair Protein Causes Dissociation of the ERCC1-XPF Complex. J. Biol. Chem. 290:20541-20555 (2015)

Karagöz, G.E. et al. Hsp90-Tau complex reveals molecular basis for specificity in chaperone action. Cell 156:963-974 (2014)

Khan, F. et al. Structural basis of nucleic acid binding by Nicotiana tabacum glycine-rich RNA binding protein: implications for its RNA chaperone function. Nucleic Acid Res. 42:8705-8718 (2014)

UV light, ionizing radiation, mutagenic compounds, and cellular metabolism are important contributors of DNA damage in cells. Of particular importance is the mammalian nucleotide excision repair (NER) pathway and associated gene products, which coordinate the elimination of UV radiation induced DNA lesions like CPDs and 6-4 photoproducts. The NER pathway entails a collective action of at least 25 proteins that are assembled at the DNA lesion. Two proteins, ERCC1 and XPF, form a very stable complex XPF-ERCC1 and function as a structure specific DNA endonuclease that nicks the damaged DNA strand at the 5' end during nucleotide excision repair. The proper functioning of this complex critically depends on the formation of a heterodimer via their C-terminal tandem helix-hairpin-helix (HhH)2 domains (Tripsianes et al., Structure 2005).

A ERCC1 point mutation F231L, located at the hydrophobic interaction interface of this complex, leads to a severe NER pathway deficiency. We analyzed the structure and stability of the complex of the F231L mutant ERCC1-XPF complex and compared it to wildtype. We showed that while the mutant heterodimer preserves the overall fold of the ERCC1-XPF (HhH)2 complex as well as its DNA binding properties, the mutation causes a side chain reorientation for residue 231 that disrupts interactions with nearby amino acids of XPF. We showed that this small, local disturbance in ERCC1-XPF heterodimer leads to a lower stability of the complex due to an increased dissociation rate (Faridounnia et al, JBC 2015).

XPF can also form a homodimeric complex, even more stable than the ERCC1-XPF complex (Das et al, Proteins 2008). Detailed biophysical analysis now showed that the ERCC1-XPF complex dissociates very rapidly. Despite this rapid dissociation XPF preferentially re-associates with ERCC1, and XPF only forms homodimers in the absence of ERCC1. We found that the structures of XPF in the homodimer and in the heterodimer differ. Only XPF just dissociated from ERCC1 has the proper conformation to re-associate with ERCC1, whereas a structural transition is required for homodimerization.

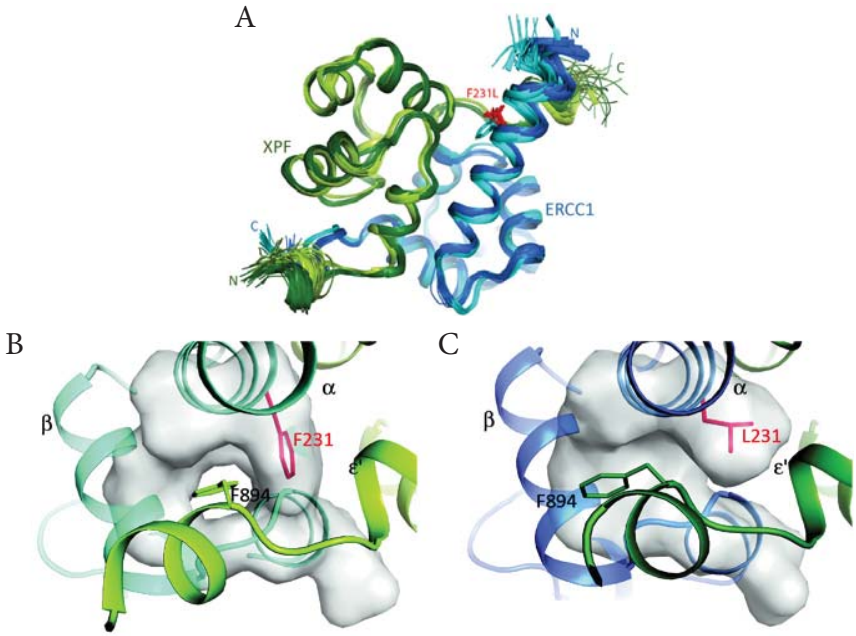


Figure: A. Solution structures of wildtype (lightblue) and F231L (dark blue) ERCC1-XPF (HhH)2 heterodimers. Part of the interaction interface of wildtype (B) and F231L mutant (C) ERCC1-XPF, showing the disruption of the F894 cavity that causes the instability of the F321L mutant complex.

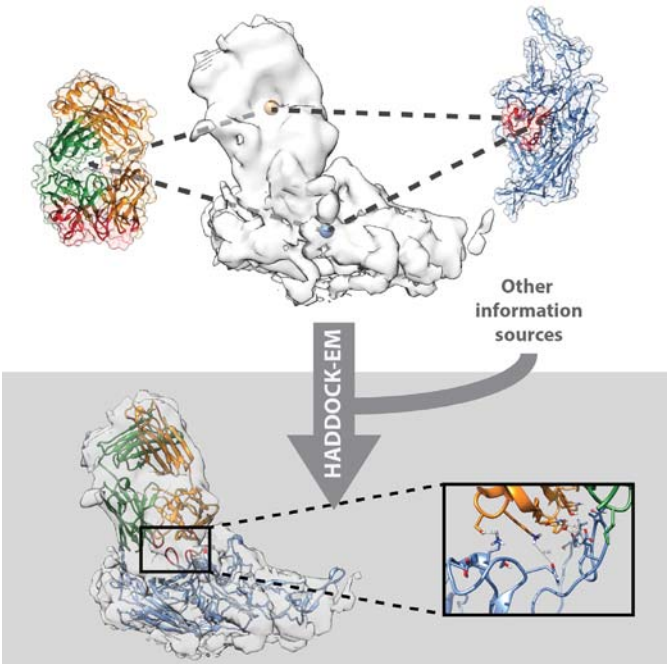
Alexandre Bonvin

In the last year, we have worked towards extending HADDOCK's capabilities for integrative modeling by adding in particular support for cryo-EM data into it. In spite of all the spectacular advances in the field leading to several atomic resolution cryo-EM structures in the last year, the resolution of most cryo-EM density maps is still too low for de novo model building. To overcome this, the data are often combined with high-resolution atomic structures. Typically, the first step in the modeling process is placing the subunits in the density as a rigid body. We have developed for this purpose PowerFit, a Python package and program for fast and sensitive rigid body fitting. It introduces a novel, more sensitive scoring function, the core-weighted local cross correlation. We further improved the search algorithm by using optimized rotational sets, by limiting the cryo-EM data size through resampling and trimming the density. Through these advances, a fine-grained rotational search can now be performed within minutes on a CPU and seconds on a GPU. PowerFit is free software and can be downloaded from https://github.com/haddocking/powerfit

Such fitting approaches, however, typically do not include information from other experimental sources nor a proper physico-chemical description of the interfaces. We have therefore implemented cryo-EM restraints into our integrative modelling platform HADDOCK, which allows for their combination with a variety of other experimental or bioinformatics data and a proper refinement of the interfaces. The resulting models have high-quality interfaces, revealing novel details of the interactions.

In parallel, we have kept working on further developing our understanding of the energetics of proteins association. We showed that the number of interfacial contacts at the interface of a protein-protein complex correlates with the experimental binding affinity. This information, combined with properties of the non-interacting surface which we have previously shown to influence binding affinity, has led to one of the best performing binding affinity predictor reported so far.

Our HADDOCK web server has been updated to version 2.2 (van Zundert et al. 2016. J. Mol. Biol. 428:720-725) (http://haddocking.org) and has reached over 7000 registered users worldwide.



Alexandre Bonvin (1964) studied Chemistry at Lausanne University, Switzerland and obtained his PhD at Utrecht University in the Netherlands (1993). After two post-doc periods at Yale University (USA) and the ETHZ (CH) he joined Utrecht University in 1998 where he was appointed full professor of computational structural biology in 2009. In 2006, he received a prestigious VICI grant from the Dutch Research Council. He has coordinated the WeNMR e-Infrastructure project, is participating to several H2020 EU projects (West-Life VRE, BioExcel, INDIGO-Datacloud) and is leading the MoBrain Competence Center under EGI-Engage. Research in his group focuses on the development of reliable bioinformatics and computational approaches to predict, model and dissect biomolecular interactions at atomic level. His work has resulted in over 180 peer-reviewed publications.

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Selected publications

van Zundert, G.C.P. et al. Fast and sensitive rigid-body fitting into cryo-EM density maps with PowerFit. AIMS Biophysics 2:73-87 (2015).

van Zundert, G.C.P. et al. Integrative modeling of biomolecular complexes: HADDOCKing with Cryo-EM data. Structure 2:949-960 (2015).

Vangone, A. et al. Contacts-based prediction of binding affinity in protein-protein complexes. eLife 4:e07454 (2015).

Geert-Jan Boons

Geert-Jan Boons was recruited in 2014 to Utrecht University from the Complex Carbohydrate research Center (UGA, USA) where he was the UGA Foundation Distinguished Professor in Biochemical Sciences. At Utrecht University, he is Professor and Chair of the Department of Biological Chemistry & Drug Discovery and is tasked to build a world leading department focused on chemical glycoscience. He has published ~245 articles, many of which have appeared in leading journals, resulting in an h-index of 52. He has received many awards and visiting professorships for contributions to glycoscience, and examples include the Claude S. Hudson Award by the Division of Carbohydrate Chemistry of the American Chemical Society (2015), and the Arthur C. Cope Career Scholars Award by the American Chemical Society (2016). He is a Steering Committee Member of Consortium for Functional Glycomics (CFG), and group leader for the glycan synthesis and glycan microarray subgroup of the CFG. Recently, he was awarded a prestigious TOP-PUNT grant from NWO.

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Selected publications

Liu, L. et al. Synthetic Enterobacterial Common Antigen (ECA) for the Development of a Universal Immunotherapy for Drug-Resistant Enterobacteriaceae. Angew. Chem.-Int. Edit. 54:10953-10957 (2015)

Wang, Z. et al. A general strategy for the chemoenzymatic synthesis of asymmetrically branched N-glycans. Science 341:379-383 (2013)

Li, X. et al. Preparation of well-defined antibody–drug conjugates through glycan remodeling and strain-promoted azide–alkyne cycloadditions. Angew. Chem. Int. Ed. 53: 7179-7182 (2014)

Geert-Jan Boons started his new research group at Utrecht University in 2015, as part of both the Utrecht Institute for Pharmaceutical Sciences and the Bijvoet Center for Biomolecular Research.

His research program seamlessness integrates method development for complex oligosaccharide and glycoconjugate synthesis, application of the new methods for the preparation of biologically important target compounds such as tumor-associated antigens, capsular polysaccharides, lipopolysaccharides, and heparan sulfates, and innovative use of the resulting compounds in biological studies. The synthetic methods developed by Boons are widely employed by others thereby providing much greater access to this important class of compound. Biological studies by the Boons group have provided insight into infectious and immunological processes and the importance of these studies are underscored by the fact that several compounds developed by his team are entering clinic evaluation.

The Boons group has designed, chemically synthesized and immunologically evaluate fully synthetic carbohydrate-based multi-component vaccines to overcome the poor immunogenicity of tumor-associated carbohydrate and glycopeptide antigens. These studies have provided a firm foundation for a clinical trail to prevent reoccurrence of breast and other types of cancers after traditional treatments such as surgery, radiation and chemotherapy.

Boons is a pioneer in the development of convergent strategies for complex oligosaccharide assembly, which make it possible to synthesize large collections of compounds with a minimal effort for structure activity relationship studies. A highlight of such an approach was the introduction of a chemo-enzymatic strategy that has provided a library of the most complex N-glycans ever synthesized. The approach makes it possible, for the first time, to prepare representative collections of cell surface glycans for the development of the next generation of glycan microarray that can probe the importance of glycan complexity for biological recognition. Furthermore, they were the first to employ chiral auxiliaries for the stereoselectively introduction 1,2-cis-glycosides, and this approach is significantly expanding the scope of polymer-supported oligosaccharide synthesis.

The Boons group has also made significant contributions to the development of methods for visualizing glycoconjugates of living cells. In particular, they have demonstrated that derivatives of dibenzylcyclooctyne are ideal for strain promoted cycloadditions with dipoles such as azides that can be introduced into glycoconjugates by enzymatic transformations. They have also introduced new chemical reports such as nitrile oxides and nitrones that make it possible to more efficiently label complex glycans of living cells. Using the new labelling methodology, they have established that Niemann-Pick type C (NPC) disease, which is fatal genetic disorder, is associated with a previously unrecognized defect in recycling of glycoproteins. This finding is paving the way for the development of new treatment strategy for the NPC disease.

Ineke Braakman

In a eukaryotic cell the endoplasmic reticulum (ER) mediates, controls, and checks folding and assembly of newly synthesized proteins, it is the source of membrane (proteins) for other organelles, and its size is regulated upon need. One of the proteins whose folding we study is the Cystic Fibrosis-related chloride channel CFTR (figure, left panel), an ABC-transporter with 2 membrane-spanning domains (TMDs) with 6 TM-spans each and long alpha-helical protrusions into the cytosol, and 2 cytosolic nucleotide-binding domains (NBD1 and NBD2) that hydrolyze ATP for channel activity. We radiolabel newly synthesized CFTR, disease-causing CFTR mutants or designed mutants and probe their conformations by limited proteolysis and domain-specific antibodies against all domains. The resulting biochemical conformational parameters we then correlate with functional parameters such as chloride-channel characteristics and patient information such as lung capacity. This approach has yielded a wealth of conclusions, establishing that TMD1 and NBD1 are the dominant domains, which fold and start to assemble already during synthesis. TMD1-NBD2 assembly is last and is defective in many NBD1 mutants. Although NBD1 and NBD2 only assemble when the channel is closed, NBD2 protease resistance ('foldedness') depends for most mutants (but not all) on NBD1 folding. The figure (right panel) shows the quantity of protease-resistant NBD1 and NBD2 fragments for a set of CF disease-causing mutations in TMD1 (up to D110H) or NBD1. Interesting is the finding that NBD1 and NBD2 are more protease-resistant in TMD1 mutants than in wild-type CFTR (top right corner of plot), suggesting that a properly folded/TM-packed TMD1 slightly destabilizes NBD1 through assembly with the coupling helix at the tip of the protruding cytosolic first intracellular loop ICL1. Using these assays we not only established hierarchy of domain folding and domain assembly in CFTR, conserved features in ABC-transporters, we also determined mode of action of 2 clinical drugs, Ivacaftor and Lumacaftor. These drugs were approved recently for treatment of CF and have CFTR as target. TMD1 turned out to be the target domain of both drugs, establishing the power of our assays.

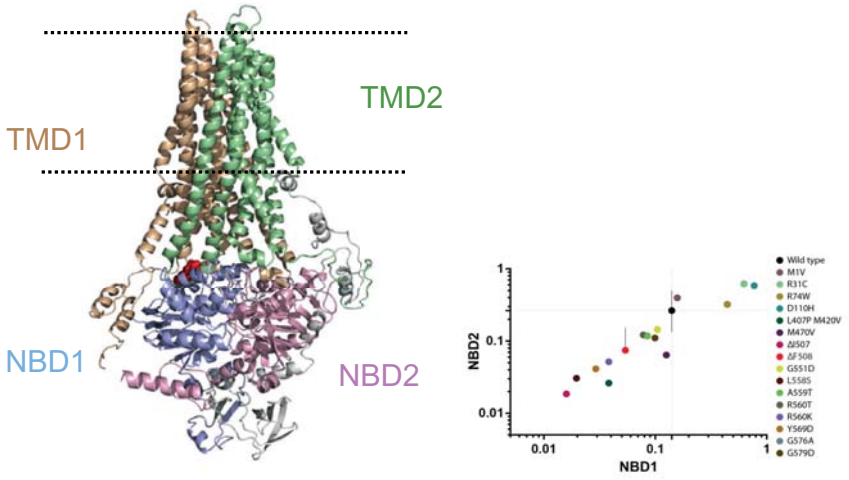

Figure: Left panel: Model of CFTR structure (Mornon et al., Cell. Mol. Life Sci. (2009) 66:3469–3486), with the 2 cytosolic nucleotide-binding domains (NBD) in blue and pink, R region in grey, and the membrane-spanning domains TMD1 and TMD2 partially in the membrane as indicated by the dotted lines. Red residue is F508, the residue most often lacking in cystic fibrosis patients. Right panel: NBD1 and NBD2 fragments were isolated from proteolysed Triton X-100 lysates of cells expressing radiolabeled full-length CFTR. Fragment quantities were plotted for a selection of disease-causing TMD1 and NBD1 mutants, including ΔF508 CFTR (red) and wild-type CFTR (black and reference lines).

Since 2000 Ineke Braakman chairs Cellular Protein Chemistry. Her research focuses on protein folding, molecular chaperones, cell stress, and organelle biogenesis and maintenance, at the interface of Chemistry, Biology, and Medicine. After a PhD in Pharmacology in Groningen, she did a post-doc in Molecular Cell Biology at Yale University, and started her group on a Fellowship from the Royal Netherlands Academy of Arts and Sciences in Amsterdam. She has been thesis advisor for 36 PhD students, delivered >200 invited seminars, (co)organized 9 international meetings, and presently is member of the 'Sectorplan Committee' for Chemistry and Physics, chair of the Program Committee of CHAINS2016, EMBO member, and she is the only European member of the 10-member North-American Cystic Fibrosis Foundation Consortium on Folding and Trafficking. Past memberships include the International Advisory Board of the IIMCB in Warsaw (2006-15), 'Commissie Dijkgraaf' (2013), the Board of the NWO Chemistry Council (2006-12), the 'College voor Promoties' (2009-12), the 'Regiegroep Chemie' (2009-11), the Board of Chemistry in Utrecht (2003-06, 2012-13), and countless evaluation panels and juries.

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Selected publications

Braakman, I. et al. De chaperonnes van eiwitten: zonder hen geen leven. P. Hoogenboom (Ed.) Natuurkundige Voordrachten 2014-2015, Nieuwe reeks no. 93. Jaarboek van de Koninklijke Maatschappij voor Natuurkunde onder de zinspreuk Diligentia, 's-Gravenhage (2015) Chapter 1.4.

Li, X. et al. Folding of influenza virus hemagglutinin in insect cells is fast and efficient. J. Biotech 203C:77-83 (2015)

Mathys, L. et al. Deletion of the Highly Conserved N-Glycan at Asn260 of HIV-1 gp120 Affects Folding and Lysosomal Degradation of gp120, and Results in Loss of Viral Infectivity. PLOS One 9:e10118 (2014)

Eefjan Breukink

Membrane Biochemistry and Biophysics

Eefjan Breukink received his PhD (in 1994) at Utrecht University. After post-doctoral research at Oxford University with Prof. A. Watts, he returned to Utrecht University, and joined the Department of Biochemistry of membranes of the Utrecht University faculty of Chemistry first as post-doc and since 2003 as faculty member. His research focuses on the bacterial cell wall synthesis pathway, determination of the mode of action of antibiotics and finding novel antibiotics in fungal extracts via smart screening techniques. He has participated in EU Networks, and (co-)authored over 100 peer-reviewed publications. Since February 2015 he is appointed as Director of education of the Bachelor Chemistry program.

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Selected publications

Gray A.N., et al. Coordination of peptidoglycan synthesis and outer membrane constriction during Escherichia coli cell division. Elife. May 7;4. doi: 10.7554/eLife.07118 (2015)

Koopmans, T. et al. Semisynthetic lipopeptides derived from nisin display antibacterial activity and lipid II-binding on par with that of the parent compound. J Am Chem Soc. 137, 9382-9389 (2015)

Mohammadi, T. et al. Specificity of the transport of lipid II by FtsW in Escherichia coli. J Biol Chem. 289:14707-14718 (2014)

With the ever-increasing prevalence of antibiotic resistance and the almost empty antibiotic pipelines of the pharmaceutical industry, there is a great need for new antibiotics. In designing new antibiotics, it is in my view best to look at the antibiotics that bacteria themselves use in their fight for survival. Evolutionary forces have shaped and continue to shape these antibiotics into excellent weapons. By learning how they work, new targets can be identified and we might be able to design better versions or even completely new antibiotics and so keep ahead in the arms race against the resistant bacteria. One of these interesting bacterial derived antibiotics is pep5, which is especially active against staphylococci (including MRSA) with MIC-values in the low nanomolar range. Unraveling its mode of action will likely teach us to specifically target staphylococci strains. The peptide acts very fast as within minutes of addition the bacteria stop growing. Yet, the action of pep5 is completely reversible as upon removal of the peptide the cells start immediately to grow again. There is also no visible effect on the morphology of the cells after treatment with pep 5 (see figure). The low nanomolar MIC value and the fact that pep5 is heavily charged (hence is highly unlikely to penetrate the membrane) point towards a specific target that is membrane-localized. We are currently trying to elucidate the mode of action of pep5, which hopefully will teach us how to tackle the multiresistant staphylococcus strains that cause large problems in hospitals throughout the world.

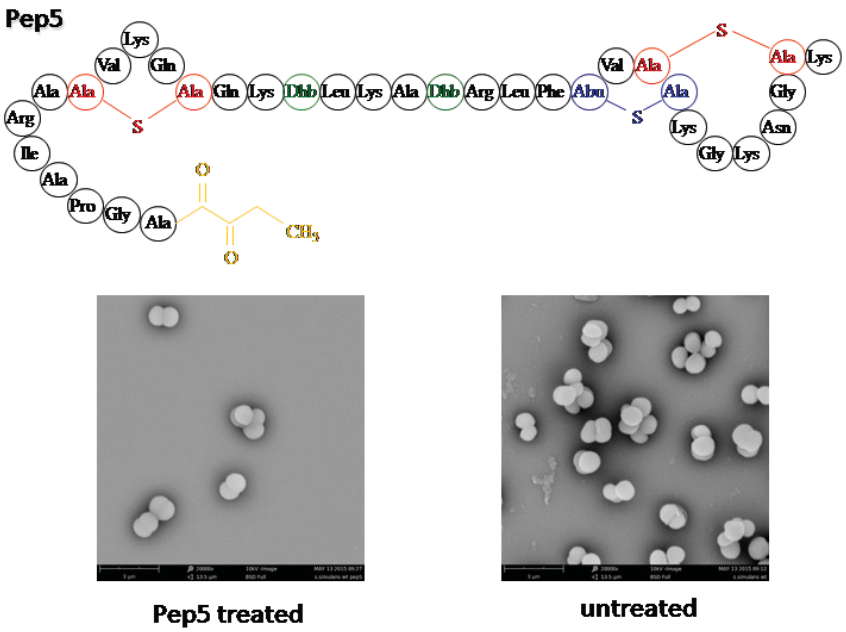


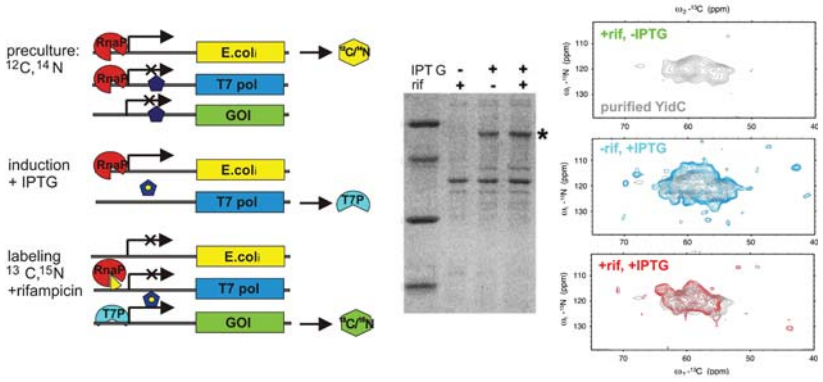
Figure: Top: primary structure of pep5, with the lanthionine residues in red, the beta-methyl lanthionine residue in blue, the dehydrobutyrine residues in green and the N-terminal 2-oxobutyryl group in yellow. Bottom: EM picture of staphylococcal cells treated with pep5 (left) or untreated (right).

Gert Folkers

For structural biology and especially for NMR spectroscopy, due to requirement for isotope enrichment, Escherichia coli is the optimal host for recombinant protein production. Over the years we implemented a high throughput protein production facility for sample preparation and streamlined screening protocols that improve both amount of protein and solubility. Such optimization strategies become increasingly important for the preparation of large amounts of protein complexes, eukaryotic multi-domain proteins or membrane proteins.

While expression and purification of membrane proteins is challenging, recent progress made in solid state NMR by us and others make it possible to study membrane with solid state NMR using membrane vesicles. This not only overcomes the requirement of membrane protein purification, generally associated with extensive losses, it also enables the analysis of proteins in the native environment. We have optimized the recombinant protein expression protocols to maximize the amount of protein produced per cell in order to improve the signal intensities in in cell NMR experiments (Baker et al). For in cell NMR both isotope enrichment and substantial overexpression is crucial. However, overexpression of membrane proteins is generally complicated. To overcome this problem we implemented a novel method to selectively label recombinant membrane proteins in E.coli by inhibiting the E.coli RNA polymerase using rifampicin which enables labeling of only the protein of interest as the T7 RNA polymerase that drives expression of the transgene is not inhibited (Baker et al). We show that we can readily detect signals from the membrane proteins in membrane vesicles after addition of rifampicin and inducer while in the absence of either of the two no signals corresponding to protein of interest was found (Fig), this approach enables us to study membrane proteins in their native environment and diminishes the signals coming from other abundant proteins present in the samples.

Pioneering studies in the group of Marc Baldus demonstrated that overexpressed bacterial outer membrane proteins can be studied at the atomic level with ssNMR. We now show that, we can, using optimized expression protocols, also characterize a megadalton, inner and outer membrane spanning, membrane protein complex in vesicles (Kaplan et al). We not only obtained residue specific information for outer membrane domain for which a structure was known but we also get structural insight into the inner membrane spanning part of the complex and show that this part adopts a different conformation than expected based on the EM data on the purified complex. Current efforts focus on the optimization of the labeling and expression methods to obtain ssNMR samples of eukaryotic membrane proteins in their native environment using cell lines that overexpress membrane proteins.



Gert Folkers did his PhD in the group of Paul van der Saag at the Netherlands Institute for developmental biology in Utrecht. In this group he worked from 1991 until 1998 on the transcription regulation by retinoid receptors. From februari 1998 he started as a postdoc in the group of prof. Rob Kaptein, to setup a laboratory for the expression and purification of proteins and to biochemically characterize proteins for which the structure has been established. In February 2005 his position was tenured and from January 2006 he is appointed as associate professor in the NMR spectroscopy section of the Science Faculty of the Utrecht University.

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Selected publications

Baker, L.A. et al. Magic-angle-spinning solid-state NMR of membrane proteins. Methods Enzymol. 557:307-28 (2015)

Baker, L.A. et al. Efficient cellular solid-state NMR of membrane proteins by targeted protein labeling. J Biomol NMR. 62:199-208 (2015)

Kaplan, M. et al. Probing a cell-embedded megadalton protein complex by DNP-supported solid-state NMR. Nat Methods. 12:649-52 (2015)

Friedrich Förster

Friedrich Förster (b. 1975) is newly appointed full Professor for Cryo-Electron Microscopy at the Bijvoet Center since early 2016. His group focuses on the structural characterization of membrane-associated and processes and those carried out by transient soluble assemblies. The work includes both development of computational methods (e.g., cryo subtomogram analysis, integrative modelling) and experimental studies of translocation of nascent mammalian proteins into the Endoplasmic Reticulum and their maturation as well as intracellular protein degradation. Förster published around 70 articles in international journals. These publications have been cited almost 5,000 times and his h-index is 33. He has received a Human Frontiers Career Development award in 2009. In recent years, his group has developed and applied a computational methodology to visualize native membrane proteins at subnanometer resolution, for example revealing the most detailed insights into the mammalian N-glycosylation machinery to date.

Contact

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Selected publications

Pfeffer, S. et al. Structure of the native Sec61 protein-conducting channel. Nature Communications 6: 8403 (2015)

Pfeffer, S. et al. Structure of the mammalian oligosaccharyl-transferase complex in the native ER protein translocon. Nature Communications 5:3072 (2014)

Unverdorben, P. et al. Deep classification of a large cryo-EM dataset defines the conformational landscape of the 26S proteasome. Proc. Natl. Acad. Sci. USA 111:5544-5549 (2014)

Longtime strengths of the group are three-dimensional (3D) cryoelectron microscopy (cryo-EM), in-novation in 3D image processing, and computational modeling of macromolecular assemblies. These cornerstones enable us to pursue an integrative approach to structural characterization of assembly function, which is particularly powerful to study weakly- and membrane-associated assemblies that are inaccessible to many other methods. We are primarily interested in the structural basis of synthe-sis, folding, post-translational modification, and degradation of secretory pathway proteins, which constitute approximately one third of the proteome of most eukaryotic cells.

Cryoelectron tomography (CET) is a versatile imaging modality that allows structural analysis of mac-romolecular complexes in their physiological microenvironment, e.g., in whole cells, purified orga-nelles or lysates. Since the resolution of a cryo-tomogram is limited by the applicable electron dose, averaging subtomograms containing identical types of macromolecules can provide much higher reso-lution insights than a single tomogram (Fig. 1A). Our broad goal is to further improve resolution of sub-tomogram averages, to enable most accurate disentanglement of different conformational states in situ, and to build atomic models that explain the observed data.

In mammalian cells secretory pathway proteins are typically co-translationally translocated across the ER membrane. The ER-translocon is an integral membrane protein complex comprising the protein-conducting channel Sec61 and several associated complexes involved in post-translational processing (e.g., glycosylation, signal peptide cleavage, and folding). We aim to study the structural details of co-translational translocation and concomitant processing in situ.

ER-residing proteins are sensitive to defects in glycosylation or disulphide bridge formation. Two mechanisms have evolved to respond to ER stress, ER associated degradation (ERAD) and the unfolded protein response (UPR), both implicated in disorders such as Alzheimer's and Parkinson's disease. We aim to image these structurally elusive processes at the molecular level using CET.

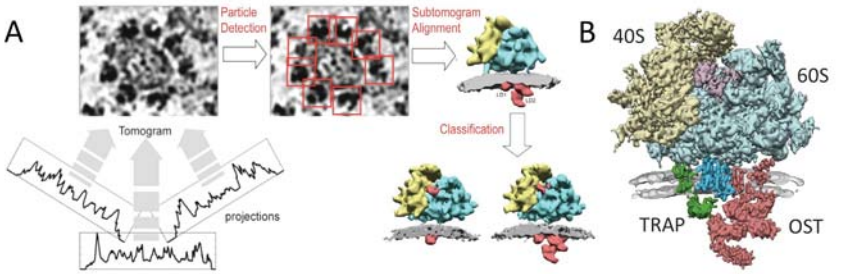

Figure: Subtomogram analysis applied to the ER-associated mammalian ribosome. A: Principle of subtomogram analysis. A tomogram of a ribosome-studded microsome is reconstructed from projections. Particles (ribosomes) are detected using automated methods and the corresponding subtomograms are aligned. Classification allows resolving differ-ent populations of particles. B: Architecture of the major complexes constituting the native translocon (protein-conducting channel Sec61, TRAP complex, and oligosaccharyl transferase complex, OST) resolved by subtomogram averaging to ~8 Å.

Willie Geerts

Traditionally transmission electron microscopy (TEM) uses ultra-thin sections of chemically fixed and plastic-embedded cells or tissues to observe the subcellular structure and membrane dynamics of organelles. Chemical fixation of samples does not perfectly preserve the ultrastructure of organelles. To circumvent this limitation, physical immobilization approaches (vitrification by high pressure freezing or plunging) have been developed. Cryo-electron microscopy tomography (cryo-ET) is becoming a mainstream technology for studying membrane architecture and high-order macromolecular complexes in their close to native cellular environment at nm resolution. The major advantage of the physical fixation method is that it immobilizes cellular structures within milliseconds, which is much faster than conventional chemical fixation procedures. In addition, over the past decade real-time imaging and 3D electron tomography have increasingly replaced classical static light microscopy imaging and 2D electron microscopy.

In my research, approaches are being developed to combine light microscopy with high-resolution (cryo) electron tomography (Correlative Light and Electron Microscopy, CLEM). CLEM utilizes complementary visual techniques that allow capturing dynamic cellular processes by (immuno) fluorescence and then zooms in on these events to identify their ultrastructure by EM tomography. 3D reconstruction techniques allow snap-frozen structures to be reassembled visually into 3D models thereby providing detailed z-axis information. To this end, we use the latest instrumentation in FIB-SEM, cryo-EM and tomography technology, 3D reconstruction software and data analysis, all serving the needs of various themes in life sciences, including the analyses of biomolecules at the atomic level.

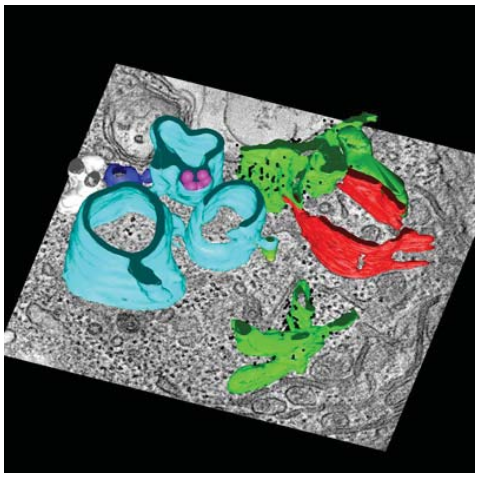

Figure: Typical example of a 3D model reconstructed from tomographic slides.

Willie Geerts obtained his HBO degree in 1978, graduated in Biology in Utrecht (1991) and received his PhD degree (1996) at the Medical faculty of the University of Amsterdam in the departments of Anatomy (Prof. Lamers) and Histology (Prof. van Noorden). In 2000 he started as a Post-doc assigned by FEI and later by the European Network of Excellence in the department of Electron Microscopy under Prof. Verkleij where he got a permanent position as assistant professor in 2006. He is specialized in 3D electron microscopical reconstruction methods. He coordinate the EM courses from the Biomolecular Imaging department and since 2010 he is the managing director of the Institute of Biomembranes.

Contact

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Selected publications

Lorenzato, C. et al. MRI monitoring of nanocarrier accumulation and release using Gadolinium-SPIO co-labelled thermosensitive liposomes. Contrast Media Mol Imaging. in press (2016)

Beznoussenko, G.V. et al. Trans-membrane area asymmetry controls the shape of cellular organelles. Int J Mol Sci. 16:5299-333 (2015)

Mari, M. et al. Immuno- and correlative light microscopy-electron tomography methods for 3D protein localization in yeast. Traffic. 15:1164-78 (2014)

Piet Gros

Piet Gros (1962) heads the laboratory of Crystal and Structural Chemistry. With his research team, he studies bio-molecular recognition and regulation processes of human plasma proteins and receptors, in particular in the area of infection and immunity. We apply molecular biology, biochemical and biophysical methods including protein crystallography and cryo-EM to resolve the molecular mechanisms underlying the key regulatory processes. Moreover, the method of ensemble refinement of crystallographic data is developed in his group. Piet Gros received an ERC Advanced Grant in 2008, was awarded the Spinoza prize 2010 by the Netherlands Organisation for Scientific Research (NWO), is a member of the Royal Academy of The Netherlands (KNAW, 2010) and of the European Molecular Biology Organization (EMBO, 2013) and was knighted in the Order of the Netherlands Lion (2013).

Contact

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Selected publications

Sharp, T.H. et al. Heterogeneous MAC Initiator and Pore Structures in a Lipid Bilayer by Phase-Plate Cryo-electron Tomography. Cell Rep. 15:1-8 (2016)

Forneris, F. et al. Regulators of complement activity mediate inhibitory mechanisms through a common C3b-binding mode. EMBO J. (2016)

Kroon-Batenburg, L.M. et al. Accounting for partiality in serial crystallography using ray-tracing principles. Acta Crystallogr D Biol Crystallogr. 71:1799-811 (2015)

Diebolder, C.A. et al. Complement is activated by IgG hexamers assembled at the cell surface. Science 343:1260-3 (2014)

In 2014-2015 the Gros-research group has continued its research into (i) membraneproteins, where we aim to determine structures of membrane proteins and of their complexes, representing activation and signal transduction processes; and, (ii) the molecular mechanisms underlying the complement system, where we focus on antibody activation through the classical pathway of complement, regulation and host protection by complement regulators in the alternative pathway and pore formation by the Membrane-Attack–Complex.

Formation of the Membrane-Attack-Complex (MAC) in membranes of bacteria is an important immune response induced by complement activation in human blood and interstitial fluids. MAC formation occurs when the terminal pathway of complement activation is initiated by cleavage of C5 into C5b, followed by sequential binding of C6, C7, C8 and multiple copies of C9, yielding C5b6789n (or C5b-9), also known as the MAC (see also our previous contributions: Hadders et al. Science 2007; Hadders et al. Cell Report 2012). In collaboration with Bram Koster's EM lab (funded by a joint NWO-CW grant), we have studied the steps of MAC formation and its effect on lipid bilayers by in situ cryo-electron tomography. In these studies, we used the Volta-phase plate installed on a Titan Krios with a Falcon II detector at NeCEN (Leiden). In particular, the ½π-phase shift introduced by the phase plate to the scattered electrons improves contrast markedly, facilitating interpretation of heterogeneous structures present in the tomographic images. Phase-plate tomographic series (Sharp et al. Cell Reports 2016) revealed extensive structural heterogeneities in MAC formation. We found variable-sized multimers for each of the C5b-7 (i.e. C5b67), C5b-8 and C5b-9 complexes on and in the membrane. Complexes of C5b-7 were seen to adhere to bilayers, bulging out the outer-leaflet, while maintaining the integrity of the membrane inner-leaflet (see Figure). In contrast, oligomeric structures of C5b-8 were seen to perforate the bilayer, forming a novel type of pore, explaining that membrane leakage observed in the absence of the ring-forming C9. C5b-9 (MAC) rings were observed forming both single pores and multimeric pores of various shapes. Reconstruction by sub-tomogram averaging yielded a 20-Å resolution structure revealing a twisted, slightly cone-shaped MAC pore in the lipid bilayer. Poor closure between the C5b-8 initiator and the C9n end yields a remarkable seam in the barrel above the membrane. Most likely, the twisted, cone-shaped barrel with its seam is due to C9 compensating for the missing, or shortened, transmembrane β-strands of C6, C7 and partly C8. Moreover, the irregular closure explains the observed variation of ring closures associated with multimeric C5b-9 pores.

In conclusion, these data indicate a dynamic process that leads to formation of MAC pores. Possibly, the variable process allows the MAC proteins to attack a variety of membranes presented by diverse pathogenic microbes.

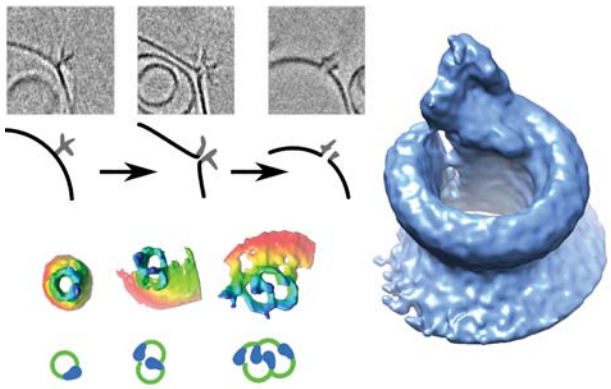

Figure: Reconstructed cryo-EM tomograms (top left) reveal stages of MAC pore formation of (from left to right) C5b-7, C5b-8 and C5b-9, each with related schematics underneath. Below are shown single and multiple C5b-9 pores, with associated schematics. Right-hand side shows a sub-tomogram reconstruction at 20-Å resolution revealing the twisted, slightly cone-shaped MAC pore in a lipid bilayer (Sharp et al. Cell Reports 2016).

Albert Heck

Native mass spectrometry

The Heck-group has distinctive expertise in the mass spectrometric analysis of intact proteins and protein assemblies. We developed, in collaboration with Alexander Makarov's team a novel mass spectrometer uniquely able to analyse large protein assemblies with very high sensitivity and mass resolution. The instrument was first described in Nature Methods. In the past year we have been focusing on the analysis of IgG hexamers and their role in Complement activation, viruses and designed protein nanocontainers. Moreover, we used native mass spectrometry to profile all proteoforms of a given protein using it for antibodies and other glycoproteins. The developed methods gain interest from the biopharmaceutical community, leading to collaborations with Roche, DSM, Genmab, and Merus.

Proteomics

Heck's work focuses on developing new mass spectrometry methods and apply such approaches in proteome studies. In 2015 we reported in Cell Reports on the highly beneficial use of multiple complementary proteases in (phospho) proteomics enlarging the detectable phosphoproteome. We also introduced a new workflow making cross-linking mass spectrometry possible in whole cell lysates. Therefore, we used a new cross-linker chemical moiety, novel peptide fragmentation techniques and identification methods and a dedicated search algorithm termed XlinkX. We described in Nature Methods this new integrated workflow that allowed us to identify cross-links from a variety of protein complexes in the human proteome. Building further on our work on hybrid fragmentation techniques, such as EThcD, we analysed not only cross-linked peptides, but also peptide epitopes presentated by MHC complexes, that can trigger our immune system. We charted hereby the peptide repertoire presented by the human leukocyte antigen (HLA) class I system and observed more than 15,000 peptides, amongst them many modified by unexpected post-translational modifications. We reported this year in JACS on such HLA peptides modified by OGlcNAc-ylation, whereby this modification had been on several peptides extended by other carbohydrate moieties, likely occurring when these peptides are on their way to the cell's surface travelling through the Golgi and ER.

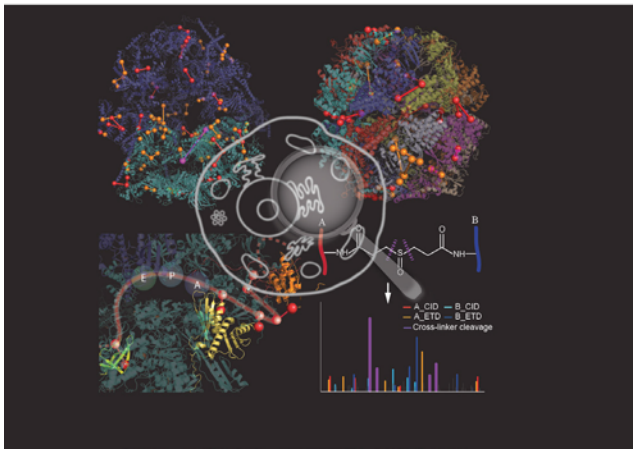

Figure: *Visualizing protein networks in the cellular environment.* Cross-linking mass spectrometry has become an important approach in structural biology of purified (over-expressed) protein assemblies. However, cross-linking could not yet be used on whole proteomes, due to the sample complexity, escalating the size of the databases to be used to identify the cross-links. Fan Liu in the group of Albert Heck reported in Nature Methods a new integrated workflow that proteome-wide identifies cross-links originating from a variety of protein complexes directly in human cellular lysates. Our approach is based on the application of a MS-cleavable cross-linker, sequential CID and ETD MS2 acquisitions, and a dedicated search engine termed XlinkX. This approach allowed us to detect 2179 unique inter-peptide cross-links in cell lysates which is by far the largest cellular cross-linking MS dataset. Intriguingly, our data revealed new structural information on several protein assemblies, captured transient/dynamic and discovered novel protein-protein interactions.

Albert Heck (1964) heads the Biomolecular Mass Spectrometry and Proteomics group. Heck is scientific director of the Netherlands Proteomics Centre, and PI of the NWO supported national roadmap facility Proteins@Work. In 2001 he was recipient of the Golden Medal of the Dutch Royal Chemical Society. Heck received numerous awards; the Descartes-Huygens Award from the Ministere de l'education nationale [2010], the Life Science Award of the German Mass Spectrometry Society [2013], the HUPO Discovery Award in Yokohama [2013], the EuPA Pioneer in Proteomics Award in Madrid [2014] and ACS Franklin & Field Award for Outstanding Achievements in Mass Spectrometry in San Diego [2016]. In 2010 he was guest-professor at the ETH Zurich. In 2013 he became PI of the Gravitation program Institute for Chemical Immunology. Heck has currently published more than 500 papers in internationally reviewed journals.

Contact

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Selected publications

Giansanti, P. et al. An Augmented Multiple-Protease-Based Human Phosphopeptide Atlas. Cell Reports 11:1834-43 (2015)

Marino, F., et al., Extended O-GlcNAc on HLA Class-I-Bound Peptides. J Am Chem Soc. 137:10922-5 (2015)

Liu, F. et al. roteome-wide profiling of protein assemblies by cross-linking mass spectrometry. Nature Methods. 12:1179-84 (2015)

Casper Hoogenraad

Associate Member

Casper Hoogenraad is head of the Cell Biology division and an expert in Molecular Neuroscience. The primary goal of this group is to get better mechanistic understand of how cytoskeleton organization and intracellular transport underlies neuronal development and synapse function. Dr. Hoogenraad performed his PhD research in the field of Molecular and Cellular Biology at the Erasmus University in Rotterdam (The Netherlands). He did a post-doc period at the Massachusetts Institute of Technology (MIT) in Cambridge (USA) after which he joined the Department of Neuroscience at the Erasmus Medical Center in Rotterdam (The Netherlands) in 2005 and received the European Young Investigators (EURYI) award, Human Frontiers Career Development Award (HFSP-CDA) and Dutch Innovative research VIDI grant. After five years of having an independent research group, Dr. Hoogenraad was promoted to Full Professor in Cell Biology at Utrecht University, and received the Dutch Innovative research VICI grant and European Research Council (ERC) consolidator grant. He is also member of the European Molecular Biology Organisation (EMBO), Young Academy member of the Dutch Royal Academy of Arts and Sciences (KNAW-DJA) and vice-chair of the Scientific Advisory Board of the Internationale Stichting Alzheimer Onderzoek (ISAO).

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Selected publications

van Beuningen, S.F. et al. TRIM46 Controls Neuronal Polarity and Axon Specification by Driving the Formation of Parallel Microtubule Arrays. Neuron 88:1208-26 (2015)

van Bergeijk, P. et al. Optogenetic control of organelle transport and positioning. Nature 518:111-4 (2015)

Yau, K.W. et al. Microtubule minus-end binding protein CAMSAP2 controls axon specification and dendrite development. Neuron 82:1058-73 (2014)

Research in the Hoogenraad group is dedicated to push the frontiers of basic and translational Neuroscience research. His research can be roughly divided in three themes: i) 'The Roadmap' - Cytoskeleton dynamics during neurodevelopment and synaptic plasticity, ii) 'Traffic Rules' - Motor proteins and adaptors as regulators of neuronal cargo transport, iii) 'Accidents on the Highway' – Brain diseases linked to the cytoskeleton and intracellular transport. The research of the Hoogenraad group relies on combining different genetic, biochemistry, molecular biology methods in in vitro (neuron cultures), ex vivo (brain slices), and in vivo (C.elegans and mice) systems. In addition, the lab uses state-of-the-art imaging techniques and applies advanced microscopy technologies.

The structural organization and dynamic remodeling of the neuronal cytoskeleton contribute to all these morphological and functional changes in neurons. Along with the actin cytoskeleton, the assembly, organization, and remodeling of the microtubule cytoskeleton are essential to successfully complete all the different stages of neuronal development. Microtubule-based motor proteins such as kinesin and dynein recognize the intrinsic asymmetry of the microtubule lattice and drive transport to either the microtubule plus-end or minus-end. In various model systems it has been shown that the microtubule arrays within axon and dendrites are highly organized with respect to their intrinsic polarity and that this specific microtubule organization is essential to direct polarized cargo transport. In addition, alterations in microtubule organization and cargo trafficking have been described in many neurodegenerative diseases. Thus, while the importance of the microtubule cytoskeleton for proper intracellular trafficking and cargo sorting is unambiguous, how the microtubule in axon and dendrites are organized in developing and mature neurons is largely unknown. We have identified some of the molecular processes that control microtubule organization and dynamics during the different stages of neuronal development. Our recent work indicates that microtubule binding proteins, such as end binding proteins (EB), CAMSAP2 and TRIM46 play an critical role in remodeling microtubules during the early stages of neuronal polarization.

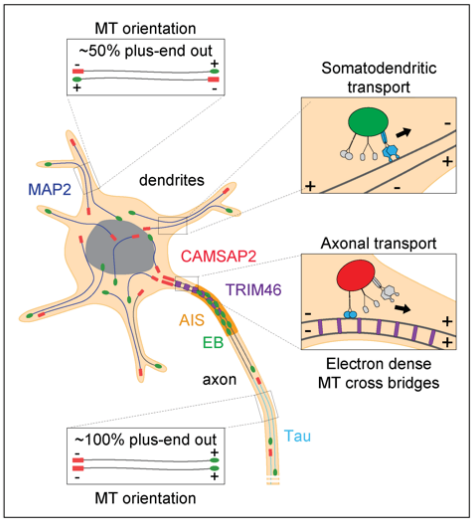

Figure: *Microtubule organization during neuronal polarization.* The microtubule cytoskeleton is a major determinant in the establishment and maintenance of neuronal polarity. Microtubules provide the structural basis for neuronal polarization, because of their intrinsic properties including inherent polarity. Moreover, the polarized microtubule network also forms the basis for selective cargo trafficking into axons and dendrites. TRIM46 localizes to the proximal axonal region, partly overlapping with the axon initial segment (AIS), and forms closely spaced plus-end out microtubule arrays with cross-bridges. The axonal microtubules are decorated by tau, whereas MAP2 is enriched in the somatodendritic compartment. The microtubule minus-end binding protein CAMSAP2, which stabilizes non-centrosomal microtubules, is enriched in the very first part of the axon, but absent from the AIS. While EB proteins are usually associated with growing MT plus ends, they have also been found to bind along the microtubule lattice in the AIS. The microtubule organization is indicated as percentages of plus-end out microtubules.

Eric Huizinga

My research focuses on structural studies of proteins involved in bleeding arrest and host-pathogen interactions. I also have a long-standing interest in optimizing recombinant expression of challenging proteins. In the latter context we developed a method coined "plasmid titration" to optimize transient transfection of HEK293 cells; the work-horse for protein production in our and many other labs. We observe that reduction of the amount of expression plasmid reduces total protein expression, but increases in many cases the amount of protein that can be purified, or, in the case of secreted proteins, the amount of protein that accumulates in the expression medium. Plasmid titration proved beneficial for studies on host-pathogen interaction involving Nod- and Toll-like receptors, two classes of pathogen recognition receptors that are notoriously difficult to express. One study focused on the pathogen Staphylococcus aureus, which secretes a range of virulence factors to evade immune recognition. One of these factors, Staphylococcal superantigen-like protein 3 (SSL3), disrupts an important component of our innate immune system namely the activation of Toll-like receptor 2 (TLR2) by bacterial lipopeptides. In collaboration with the van Strijp group at the Utrecht Medical Centre we solved the crystal structures of SSL3 and its complex with TLR2. The structure reveals that SSL3 binds near the lipopeptide binding site of TLR2. The SSL3 binding site substantially overlaps the binding site of TLR1, preventing TLR1-TLR2 dimer formation and down-stream signalling. In the complex, the size of the entrance to the lipopeptide binding pocket is reduced by the presence of SSL3. We show that this is sufficient to inhibit binding of lipopeptide Pam2CSK4 effectively, yet allows SSL3 to bind to an already formed TLR2-Pam2CSK4 complex. SSL3 also binds to preformed TLR2-phospholipid complexes, because phosphatidylcholine is present in the TLR2-lipid binding-pocket inside our crystals as was suggested by residual electron density and proven by mass spectrometry. Binding to phospholipid-engaged TLR2 may well be functionally relevant, because in vivo TLR2, like many other lipid-binding proteins, is likely to be always present in complex with a lipid of some sort. Combined, our data reveal a robust dual mechanism in which SSL3 interferes with TLR2-activation at two stages: by binding to TLR2, it blocks ligand binding and thus inhibits activation. Secondly, by interacting with an already formed TLR2-lipopeptide complex, it prevents TLR-heterodimerization and downstream signalling.

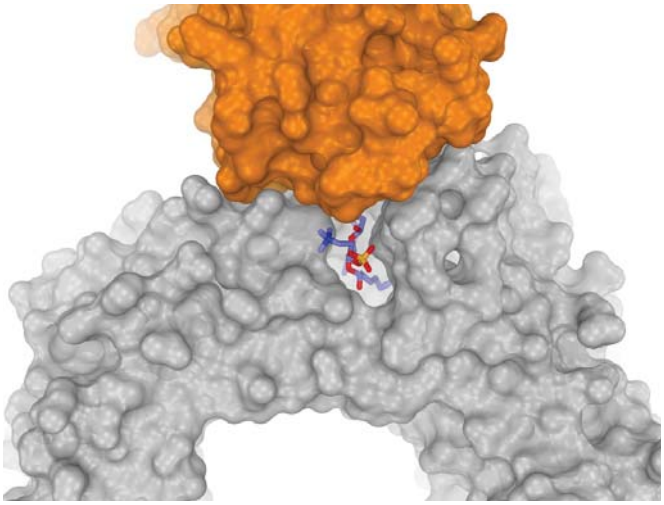

Figure: *S. aureus* virulence factor SSL3 (orange) binds close to the lipopeptide-binding site of immune receptor TLR2 (grey) thereby preventing TLR2 dimerization and signaling. Shown in sticks is a phosphatidylcholine molecule observed to occupy the lipopeptide binding pocket in our crystals.

Eric Huizinga is assistant professor in the laboratory of Crystal and Structural Chemistry. He uses protein crystallography to study adhesion and signaling in haemostasis and immunity. He has a keen interest and broad expertise in the recombinant expression of challenging proteins. He graduated in Chemistry in 1989 and received his PhD in Protein Crystallography in1994 with prof.dr. Wim Hol at the University of Groningen. After a postdoc with prof. dr. Jan Sixma in the Laboratory of Thrombosis and Haemostasis (UMC Utrecht), he moved to Crystal and Structural Chemistry in 2001 where he became assistant professor in 2002.

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Selected publications

Koymans, K.J. et al. Structural basis for inhibition of TLR2 by staphylococcal superantigen-like protein 3 (SSL3). PNAS 112, 11018-23 (2015).

Diebolder, C.A. et al. Cryoelectron Tomography of the NAIP5/NLRC4 Inflammasome: Implications for NLR Activation. Structure 23, 2349-57 (2015).

Halff, E.F. et al. When less becomes more: optimization of protein expression in HEK293-EBNA1 cells using plasmid titration - a case study for NLRs. Protein Expr Purif. 99, 27-34 (2014).

Bert Janssen

Bert Janssen obtained his PhD (2007, cum laude) in structural biology at Utrecht University. During his PhD he addressed questions central to human immunology by solving crystal structures of the large complement component C3 and its activation products C3b and C3c. From 2008 until 2011 he was a postdoctoral fellow at the University of Oxford in the lab of Prof. Yvonne Jones, funded by a HFSP fellowship. In Oxford he elucidated the molecular mechanism underlying semaphorin-plexin function in neuronal connectivity. In July 2011 he returned to Utrecht University to start his own group to work on molecular signalling processes that are critical for the homeostasis and functioning of the central nervous system. In 2012 he was awarded a starting independent researcher grant (VIDI) from the Netherlands Organisation for Scientific Research (NWO) and he became a team leader in the Marie Curie Initial Training Network "ManiFold". In 2015 he was awarded an ERC Starting Grant to further strengthen his research team.

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Selected publication

M.F. Pronker, et al. Olfactomedin-1 has a V-shaped disulfide-linked tetrameric structure. J Biol Chem 290:15092-15101 (2015)

Work in the Janssen group centers on elucidating the molecular mechanisms that underlie intercellular communication. Intercellular communication, mediated by cell-surface expressed proteins, is a fundamental aspect of the formation, function and pathology of tissues and organs. The structure of, and interactions between, extracellular regions of transmembrane proteins determine signalling and adhesion events.

In particular in our nervous system intricate intercellular connections are highly abundant. One of these neuronal intercellular signalling systems has been the subject of our research published in 2015. The protein Olfactomedin-1 is the prototypical member of an olfactomedin domain-containing glycoprotein superfamily involved in the formation and homeostasis of a broad range of tissues. Olfm1 is highly expressed in the brain and important for the development and function of the nervous system.

We have combined crystal structure determination, electron tomography and small angle X-ray scattering to show that Olfm1 forms V-shaped disulphide-linked homotetramers and that this structure is likely common to most olfactomedin superfamily members. The crystal structure of the tips of the Olfm1 V-shape provides a clear picture of the olfactomedin domains that are used to engage receptors on the neuronal membrane to induce signalling events. The V-shaped architecture is probably important for the function of Olfm1; the distinct V-shape of Olfm1 enables the molecule to bring together the engaged neuronal receptors to induce intracellular signalling events.

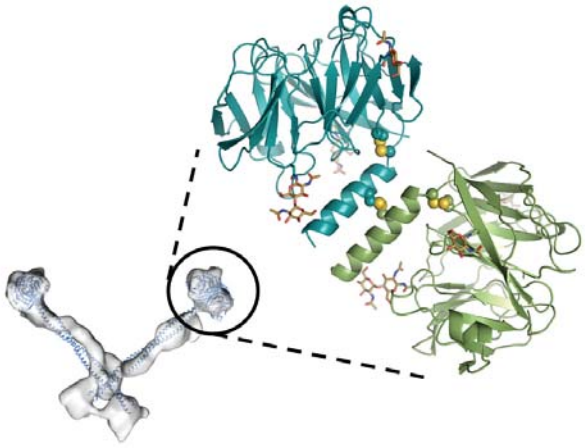

Figure: Using a combination of techniques (electron tomography, SAXS, AUC and protein crystallography) we solved the structure of Olfactomedin-1.

Antoinette Killian

Much of our research efforts of the past years were focussed on the exploration of styrene-maleic acid (SMA) polymers as novel tool in membrane research. SMA polymers are able to solubilize membranes in the form of small nanodiscs, allowing purification and characterization of membrane proteins directly in their native environment without the use of detergent.

The SMA technology was used to solubilize the tetrameric potassium channel KcsA from E.coli and the Reaction Center from Rhodobacter sphearoides. We were able to purify both protein complexes in the form of so-called "native nanodiscs" by affinity chromatography. The small size (~10 nm) and high thermostability of the nanodiscs allowed analysis of the structural and functional properties of the protein complexes by a variety of spectroscopic methods. In addition, by analysis of the lipid composition of the nanodiscs we were able to identify preferential lipid interactions of the protein complexes in their native lipid environment. Finally, for KcsA we found that it was possibe to transfer the channel from the nanodiscs directly to membranes of E.coli lipids, thus allowing characterization of channel function.

In complementary approaches we have been investigating the mode of action of SMA using model membrane systems. This allows systematic and straightforward variation of many potentially important parameters. These experiments have led to new insights into the molecular mechanism of nanodisc formation and they have helped to optimize the use of SMA for solubilization of proteins from different native membranes. In addition, we have explored entirely new applications of SMA in membrane research, such as isolation of liquid-ordered domains and analysis of helix-helix interactions in membranes.

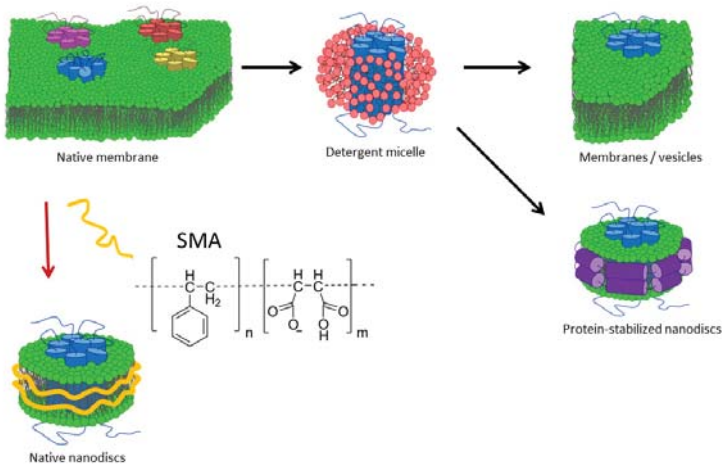

Figure: Schematic representation of ways of reconstituting membrane proteins in a bilayer environment after purification in micelles (black arrows) versus direct solubilization of membrane proteins from their native environment in the form of native nanodiscs (red arrow)

Antoinette Killian is head of the research group Membrane Biochemistry & Biophysics. Her group studies interactions of lipids with membrane proteins and membrane-active compounds by combining biochemical and biophysical techniques with chemical biology and molecular biology tools. Antoinette Killian received her PhD in 1986 at Utrecht University and did a postdoc in Alabama and Florida, financed by an NWO stipend. She then returned to Utrecht University, where she became full professor in 2002. She spent a sabbatical period at the University of Umeå in Sweden in 1993 and In 2013 she was appointed visiting professor at the Institute Pierre et Marie Curie in Paris. She received several awards for her work, including the FEBS (Federation of European Biochemical Societies) prize in 1995, an honorary doctorate degree at the University of Umeå in Sweden in the same year, the EBSA (European Biophysical Societies' Association)/Avanti award in 2015, and in 2016 she was named Fellow of the Biophysical Society. She has a total of more than 170 publications in refereed journals.

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Selected publications

Cybulski, L.E. et al. Activation of the bacterial thermosensor DesK involves a serine zipper dimerization motif that is modulated by membrane thickness, Proc Natl Acad Sci U S A. 1123:6353-6358 (2015)

Swainsbury, D.J. et al. Bacterial Reaction Centers Purified with Styrene Maleic Acid Copolymer Retain Native Membrane Functional Properties and Display Enhanced Stability. Angew Chem Int Ed Engl. 53:11803-11807 (2014)

Dörr, J.M. et al Detergent-free isolation, characterization and functional reconstitution of a tetrameric K+ channel: the power of native nanodiscs. Proc Natl Acad Sci U S A. 111:18607-18612 (2014)

Toon de Kroon

Membrane Biochemistry and Biophysics

Toon de Kroon received his PhD cum laude (1991) at Utrecht University. After post-doctoral research at Stanford University with Harden McConnell funded by an NWO-stipend, he returned to Utrecht University, and became faculty member of the Department of Chemistry in 1995. His research focuses on lipid-protein interactions and their functions in cell biology, and on the regulation of membrane lipid homeostasis. He received an NWO "Jonge Chemici" fellowship, has participated in several EU-Networks, and (co-)authored over 80 peer-reviewed publications.

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Selected publications

Furse, S., et al. Phosphatidylcholine's functions beyond that of a membrane brick. Mol. Membrane Biol. 32, 117-119 (2015)

Renne, M.F. et al. Lipid acyl chain remodeling in yeast. Lipid Insights 8(S1), 33-40 (2015)

Marqués-Gallego, P. et al. Ligation strategies for targeting liposomal nanocarriers. BioMed. Res. Int. 2014, 129458 (2014)

Membrane contact sites are increasingly being recognized as intracellular hubs crucial for inter-organelle signal transduction, organelle morphology and the regulation of membrane lipid composition. Recently, plasma membrane - endoplasmic reticulum contact sites have been implicated in the regulation of phosphatidylcholine synthesis. Phosphatidylcholine (PC) is the most abundant membrane lipid in most eukaryotes and is synthesized via two different pathways. The Kennedy pathway requires choline, which after activation to CDP-choline merges with diacylglycerol to yield PC. The other route involves the triple methylation of phosphatidylethanolamine (PE) with mono- and dimethylated PE intermediates, PMME and PDME, respectively, and uses S-adenosyl methionine (SAM) as methyl donor. In yeast two distinct methyltransferases, Cho2p and Opi3p, both integral membrane proteins of the ER, catalyze PE methylation. Yeast mutants with impaired ER-plasma membrane contact sites were shown to be choline auxotrophs and exhibit reduced Opi3p activity. Based on these and other results we proposed that Opi3p is capable of in trans inter-membrane catalysis, methylating substrates in the juxtaposed plasma membrane. This hypothesis has been tested in in vitro systems that are based on the conversion of liposomal lipid substrates by Opi3p localized in isolated ER membranes and by in vitro synthesized Opi3p that is co-translationally assembled into nanodiscs. The membrane topology of Opi3p was solved by cysteine scanning and found to be consistent with trans-catalysis with the localization of the putative active site in the cytoplasm. The amino acids involved in the catalytic cycle of Opi3p were identified by homology modeling. Isoprenylcysteine carboxyl methyltransferase (ICMT) shows high similarity to Opi3p in its C-terminal region and good overall structure overlay (see figure). Moreover amino acids forming hydrogen bonds with SAM in ICMT are conserved in Opi3p (D128, P149, Y151, E188). Alanine substitution of each of these residues abrogates Opi3p activity.

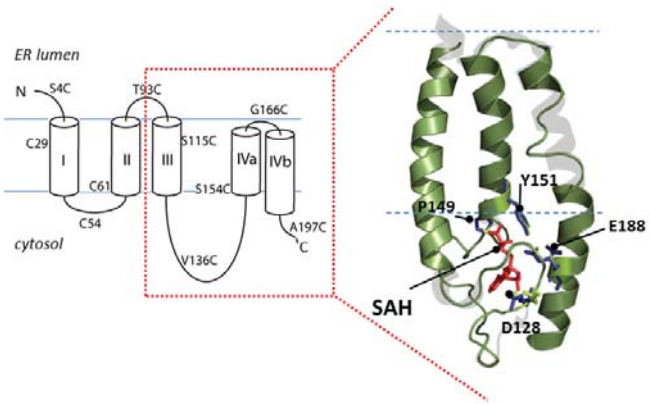

Figure: Membrane topology of Opi3p (with the cysteine substitutions made), and structure prediction of its C-terminal part based on homology modeling using the known structure of isoprenylcysteine carboxyl methyltransferase from M. acetivorans (in grey) with bound S-adenosyl-homocysteine (SAH).

Loes Kroon-Batenburg

The main theme of the research work is the development of methods for accurate integration of diffraction data. All methods are implemented in the software suite EVAL. Diffraction data can be from crystals of small inorganic/organic compounds upto large molecular complexes. Serial (femtosecond) crystallography is an exciting new development in the field, where micro- or nano-crystals are exposed to ultra brilliant X-ray laser beams during only a few femtoseconds. The diffraction patterns have to be recorded before the crystal explodes due to radiation damage. Crystals are delivered through injection devices that spray crystal suspensions across the beam interaction point. Thus the data comprise thousands upto millions of still snap shots. The challenge is to index the patterns, and thus find the crystal orientation for each, and to correct for the partial nature of the diffracted intensities. We adapted our EVAL15 software suite to treat such still snap shot data, and developed the theory and procedure for retrieving the correct intensities. We used the EVAL ray tracing method, that models the diffraction process and a logical outcome of this is the reflection partiality. We have shown, by comparing with the standard rotation data set of HEWL measured on our home source and diffractometer, how the data have to be corrected and that significant improvement can be obtained compared to the commonly used Monte Carlo integration. One of the findings is that the reflection intensities have to be corrected for a still Lorentz factor, and issue that was under strong debate. We are currently proceeding with real XFEL data, which obviously have to be modelled with different beam and sample characteristics.

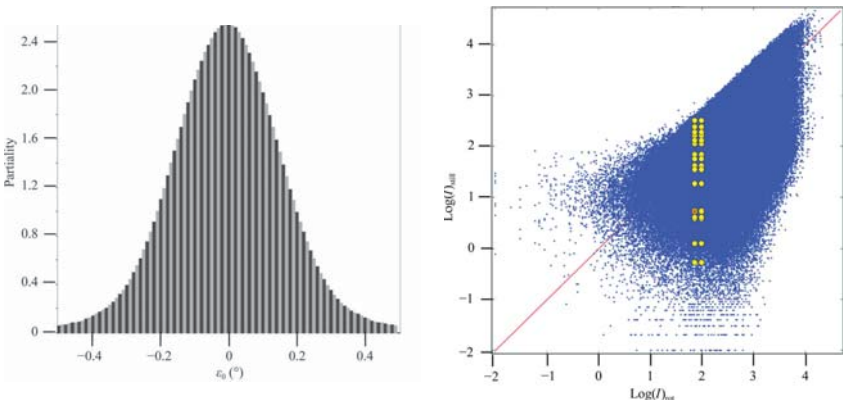

Figure: Partiality distribution of still diffraction data versus the Bragg off-set angle (left) and comparison of still and rotation type data (right). The yellow dots indicate the spread in intensity found for a single unique reflection, before applying partiality correction.

Loes Kroon-Batenburg (1956) works in an area in between Structural Biology and Material Science at the laboratory of Crystal and Structural Chemistry. Her main research project concerns the development of data collection and processing techniques for X-ray diffraction. The EVAL15 software package is freely available to the academic community. Recent developments are towards understanding and using diffuse scattering in probing internal dynamics in proteins and processing of X-ray Free Electron laser serial femtosecond diffraction data. She is member of the IUCr (International Union of Crystallography) Diffraction Data Deposition Working Group and is member of the International Program Committee for the IUCR2017 international meeting in Hyderabad.

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Selected publications

Kroon-Batenburg, L.M.J. et al. Accounting for partiality in serial crystallography using ray-tracing principles. Acta Cryst. D 71:1799-1811 (2015)

Kroon-Batenburg, L.M.J. et al. Experiences with making diffraction image data available: What metadata do we need to archive? Acta Cryst. D 70:2502-2509 (2014)

Elgersma, R.C., et. al. PH-controlled aggregation polymorphism of amyloidogenic Aβ (16-22): Insights for obtaining peptide tapes and peptide nanotubes, as function of the N -terminal capping moiety. European Journal of Medicinal Chemistry. 88:55-65 (2014)

Simone Lemeer

Biomolecular Mass Spectrometry and Proteomics

Simone Lemeer studied Chemistry at Utrecht University. After graduating she started a PhD in the Biomolecular Mass Spectrometry and Proteomics group of Albert Heck. During her PhD project, a collaboration between Utrecht University and the Hubrecht Institute, she used mass spectrometry to study phosphorylation and signaling during embryonic development in the zebrafish. In 2009 she moved to the Proteomics and Bioanalytics group of Bernhard Kuster at the Technical University Munich, Germany where she became group leader proteomics in 2011. Her work in Munich focused on the development of chemical proteomics and phosphoproteomics methods and their application in cancer research. In January 2014 she returned to Utrecht, where she became assistant professor. In the same year she reveiced a NWO VIDI grant. Her work focusses on resistance mechanisms in cancer cells studied by chemical and phosphoproteomics.

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Selected publications

Ruprecht, B. et al. Comprehensive and reproducible phosphopeptide enrichment using Fe-IMAC columns. Mol Cell Proteomics 14:205-15 (2015)

Wilhelm, M. et al. Mass-spectrometry-based draft of the human proteome. Nature 509:582-7 (2014)

My research focuses on the development and implementation of mass spectrometry based proteomic techniques to study post-translation modifications of proteins, such as phoshophorylation, to elucidate aberrant signal transduction pathways in cancer cell biology. Research in my group is focused on the both the development of analytical tools as well as the application of these tools to study deregulated signaling pathways. Here the development and implementation of the Fe-IMAC column format for the comprehensive enrichment of phosphopeptides from complex samples was a major achievement in the last years. Our offline approach was shown to be both robust and flexible and offers selective, comprehensive and reproducible enrichment and scales over wide range of sample quantities. We showed that this method outperforms all other currently applied methods such as Ti-IMAC and TiO2 enrichment. In addition our data argues that the often quoted apparent orthogonality of all three methods is caused by a combination of format inadequacies, inefficient elution and insufficient data acquisition speed rather than exploiting different physicochemical characteristics of phosphopeptides. Another highlight in the last 2 years was the publication of the first mass spectrometry draft of the Human Proteome. This publication got a lot of attention from the media as well as from the scientific community.

Supported by a VIDI grant (2014), we are currently investigating how cancer cells evade drug treatment at an early onset of treatment. Constitutive activity of kinases is known to be crucial for a tumor to maintain its malignant phenotype. Small molecule inhibitors are therefore attractive therapeutics as they target these kinases that are critical for tumor cell survival and proliferation. Several small molecule inhibitors have been proven successful in the clinic, but despite initial success most cancers eventually develop resistance against these drugs. By implementing our Fe-IMAC enrichment strategy en by using chemical proteomics tools to study kinases directly, we found that cancer cells rewire their signaling pathway and their expression to evade drug treatment before becoming resistant and regaining growth capacity.

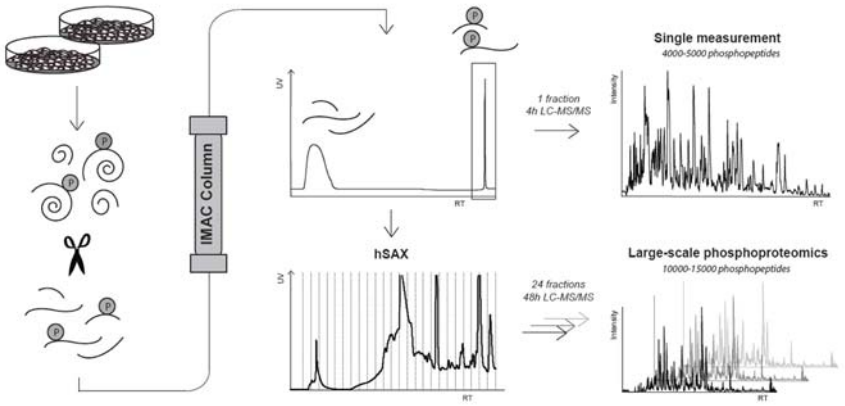

Figure: Workflow utilizing a Fe-IMAC column HPLC setup for the purification of phosphopeptides. For high throughput applications phosphopeptides are directly measured whereas subsequent hSAX separation provides increased analytical depth.

Martin Lutz

The National Single Crystal X-ray Facility now offers for external groups the crystallization of proteins and the X-ray structure determination of protein crystals. This gives new possibilities, for example in the field of fragment based drug development. In 2015 the first successful collaboration in this field was started together with a Dutch biotech company. In chemical crystallography the intense work has been continued at a high quality standard. 59 projects have been started, of which 52 have been successfully finished. The projects were mainly from Utrecht University because one of our main collaborators at the University of Amsterdam is now operating his own X-ray diffractometer. Our X-ray facility is collaborating with the crystallography group at the Radboud University in Nijmegen. Together we offer a shared entry point (http://www.ChemX.nl) for all crystallographic questions (single crystal and powder diffraction, crystallization, database research, etc.). Also, the X-ray Facility was interacting closely with the group for crystallographic methods (L. Kroon-Batenburg) in our own laboratory. In 2015, the work of the X-ray Facility resulted in 22 contributions to scientific publications. They were published in high standard chemical journals, among which were Angew. Chem., Chem. Commun., and Chem.-Eur.J. By adding protein crystallography as an additional item to the research portfolio, the National Single Crystal X-ray Facility is well prepared for future challenges.

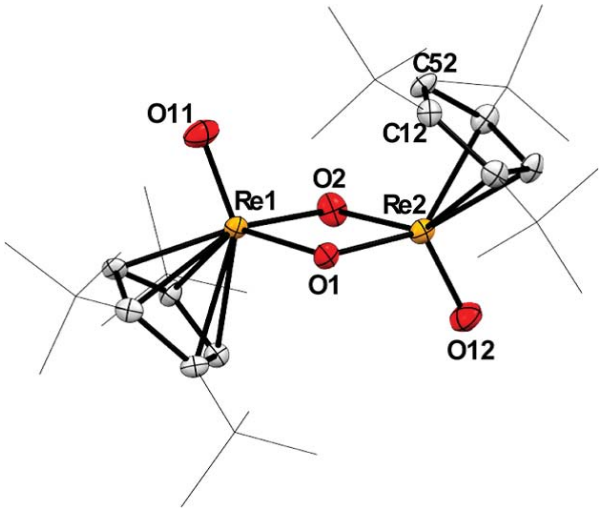

Figure: Characterization of a Rhenium(V) dioxo complex by single crystal X-ray structure determination. This complex can play an important role in the conversion of cellulosic biomass to chemically useful compounds. See S. Raju, J.T.B.H. Jastrzebski, M. Lutz, L. Witteman, J.R. Dethlefsen, P. Fristrup, M.-E. Moret, R.J.M. Klein-Gebbink (2015). Inorg. Chem. 54, 11031-11036.

Martin Lutz studied chemistry at the University of Konstanz, Germany, and received his diploma in 1992. In 1997 he obtained his Ph.D. degree under the supervision of Gerhard Müller about structural chemical investigations on complexes of main group metals (Konstanz, Germany). From 1997-1999 he has been a post-doc at Utrecht University in the group of Crystal and Structural Chemistry with prof. Anthony L. Spek. He continued as a researcher in the National Single Crystal X-ray Facility and since 2009 he is head of the facility.

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Selected publications

Vreeken, V. et al. C-H Activation of Benzene by a Photoactivated Ni-II(azide): Formation of a Transient Nickel Nitrido Complex. Angew. Chem.-Int. Edit. 54:7055-7059 (2015)

Borger, J.E. et al. Functionalization of P-4 Using a Lewis Acid Stabilized Bicyclo-[1.1.0]tetraphosphabutane Anion. Angew. Chem.-Int. Edit. 53:12836-12839 (2014)

Broere, D.L.J. et al. Intramolecular Redox-Active Ligand-to-Substrate Single-Electron Transfer: Radical Reactivity with a Palladium(II) Complex. J. Am. Chem. Soc. 136:11574-11577 (2014)

Nathaniel Martin

Nathaniel obtained his PhD degree in 2004 from the University of Alberta. As a PhD student he investigated the structures and modes of action of bacteriocins (bacterially produced antimicrobial peptides). After completing his studies in Alberta he moved to the University of California, Berkeley to pursue postdoctoral studies. In 2007 Nathaniel was awarded an NWO-VENI grant followed by an NWO-VIDI grant in 2010 providing the opportunity to initiate his independent research programme at Utrecht University. As an associate professor in the Chemical Biology & Drug Discovery group Nathaniel supervises a team of researchers whose interests involve the development of new methods for the synthesis of biologically active peptides and small molecules. These approaches are generally applied towards developing new (bio)chemical approaches for combatting infectious disease and developing new inhibitors and molecular tools with which to study cancer and epigenetic processes.

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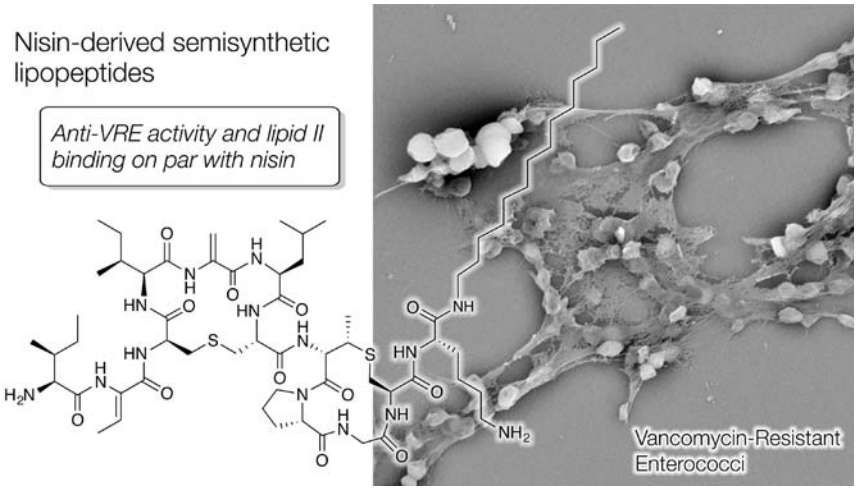
Selected publications

Kleijn, L.H.J. et al. Total Synthesis of Laspartomycin C and Characterization of its Antibacterial Mechanism of Action. Journal of Medicinal Chemistry in press (2016)

Koopmans, T. et al. Semisynthetic lipopeptides derived from nisin display antibacterial activity and lipid II-binding on par with that of the parent compound. Journal of the American Chemical Society, 137:9382–9389 (2015)

van Haren, M. et al. Synthesis and evaluation of protein arginine N-methyltransferase inhibitors designed to simultaneously occupy both substrate binding sites. Organic and Biomolecular Chemistry 13:549-560 (2015)

One of the major research themes in the Martin group is the development of new classes of (semi)synthetic antibiotics capable of selectively targeting and killing bacterial cells. To do so we apply the tools of both synthetic chemistry and chemical biology to develop novel antimicrobial compounds that interfere with bacterial cell wall biosynthesis. To date we have successfully discovered a number of new compounds that kill bacterial cells via a variety of novel mechanisms. Ongoing work is aimed at optimizing the activity of these "lead compounds" and fully characterizing their modes of action using various biochemical and biophysical techniques. As a specific example, we recently developed a series of novel semi-synthetic lipopeptide antibiotics (see figure), derived from the naturally occurring antimicrobial peptide nisin (J. Am. Chem. Soc. 2015, 137, 9382–9389). These new lipopeptide antibiotics display potent antibacterial activity against vancomycin-resistant bacteria on par with that of currently used last resort antibiotics. The key feature of these semi-synthetic antibiotics is their antibacterial mechanism of action, which is completely different than any clinically used antibiotic. Taken together these compounds are highly promising candidates for further development as new antibiotics capable of overcoming resistance in bacteria.



Madelon Maurice

The WNT/β-catenin signaling cascade is essential for proper organismal development and is frequently misregulated in cancer. A major research theme in the Maurice group is to understand the molecular mechanisms by which cells interpret WNT signals received at their cell surface and how dysregulation of these events by mutations leads to disease. By uncovering molecular details of how the subcellular localization, complex formation and activity of WNT pathway proteins direct cellular decisions we aim to provide novel clues to modulate WNT-mediated cellular responses.

A recent highlight of our work is the discovery that mutations in the WNT receptor LRP6 cause oligodontia, a severe form of tooth agenesis. This work was done in collaboration with Gjis van Haaften and Marie-José van den Boogaard, Medical Genetics, UMC Utrecht. Through exome sequencing on 20 unrelated individuals with apparent non-syndromic oligodontia, heterozygous variants in LRP6 were detected, and sequencing of additional oligodontia-affected individuals yielded one additional mutation in LRP6. Three mutations (c.1144_1145dupAG [p.Ala383Glyfs*8], c.1779dupT [p.Glu594*], and c.2224_2225dupTT [p.Leu742Phefs*7]) are predicted to truncate the protein, whereas the fourth (c.56C>T [p.Ala19Val]) is a missense variant of a conserved residue located at the cleavage site of the protein's signal peptide. All four affected individuals harboring a LRP6 mutation had a family history of tooth agenesis. LRP6 encodes a transmembrane cell-surface protein that functions as a co-receptor with members from the Frizzled protein family in the canonical Wnt/β-catenin signaling cascade. We show that the LRP6 missense variant (c.56C>T) results in altered glycosylation and improper subcellular localization of the protein, resulting in abrogated activation of the Wnt pathway. Our results identify LRP6 variants as contributing to the etiology of non-syndromic autosomal-dominant oligodontia and suggest that this gene is a candidate for screening in DNA diagnostics.

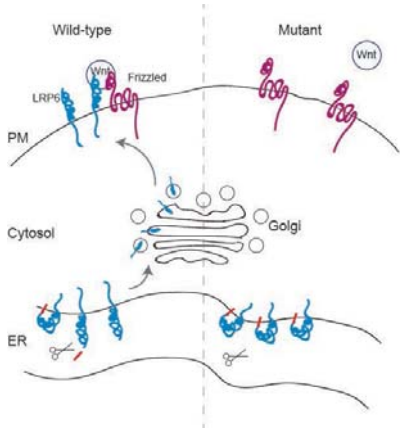


Figure: *Model: Defective signaling capacity of mutant Lrp6 p.Ala19Val is due to ER retention.* In contrast to wild-type Lrp6 (left), mutant Lrp6 p.Ala19Val fails to reach the cell surface due to defective signal peptide cleavage (right). A decreased number of Lrp6 receptors at the cell surface compromises Wnt signal transduction and leads to oligodontia in human patients. ER, Endoplasmic Reticulum, PM, plasma membrane

Madelon Maurice graduated in Medical Biology at the Free University, Amsterdam (1993). She earned her PhD in 1998 at the Department of Rheumatology, LUMC (Leiden). From 1998 – 2001, she was a postdoctoral fellow at Harvard Medical School, Boston, USA, in the lab of Hidde Ploegh. In 2001, she moved back to perform a post-doc in the lab of Hans Clevers, Hubrecht Institute, Utrecht. In 2006, she was appointed as an assistant professor at the Department of Cell Biology, UMC Utrecht, and became tenured associate professor in 2012. Her research focuses on: Molecular mechanisms of Wnt signalling in development and cancer. Her work is supported by a UU High Potential grant (2006), an ERC Starting Grant (2009), an ERC Proof of Concept Grant (2012) and an NWO Vici grant (2015).

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Selected publications

Zlatanou, A. et al. USP7 is essential for maintaining Rad18 stability and DNA damage tolerance. Oncogene 35:965-976 (2016)

Massink, M.P. et al. Loss-of-Function Mutations in the WNT Co-receptor LRP6 Cause Autosomal-Dominant Oligodontia. Am J Hum Genet. 97:621-626 (2015)

Gerlach, J.P. et al. Wnt signalling induces accumulation of phosphorylated β-catenin in two distinct cytosolic complexes. Open Biology 4:140120 (2014)

Wally Müller

Wally Müller obtained his PhD on the thesis "Localization of penicillin biosynthesis in Penicillium chrysogenum" in 1991 from Utrecht Universiteit (UU). Between 1992 – 2000 he worked as a post-doc at the UU with Unilever Research (URL) Vlaardingen and with the Centraal bureau van Schimmelcultures (CBS) Utrecht. From 2001 to 2012 he worked as an assistant professor at the Department Biology of the UU with DSM-gist Delft and CBS with research interests on the subcellular architecture in yeast and filamentous fungi. Since December 2012 he has been working at the Department of Chemistry, Cryo-EM group, with professor dr. ir. Hans Gerritsen UU and Professor dr. Judith Klumperman UMC on an integrated microscopy project as part of the STW - perspective "Microscopy Valley" research.

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Selected publications

Wang, F. et al. FluG affects secretion in colonies of Aspergillus niger. Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology 107:225-40 (2015)

Krijgsheld, P. et al. Deletion of flbA results in increased secretome complexity and reduced secretion heterogeneity in colonies of Aspergillus niger. Journal of Proteome Research 12: 1808-1819 (2013)

Previous work about resin embedding of cultured cells has shown in three different ways, cells are embedded in a resin block; cells are resin embedded in such way that the cells contours are seen; or cells are ultra-thin resin embedded resulting in the visualization of single cells as well as detailed cell surface structures. After these three different embedding methods the cells can be sectioned to show how the interior of the cell looks like in a dual-beam, i.e. a focused ion beam to section and a scanning electron microscope to visualize. However in the three embedding methods a protective layer of platinum is needed to prevent cell damage during ion-beam milling. When cells are labelled with gold particles of about 20 nm to show the location of specific proteins on the cell surface, these gold particles will be snowed under by the platinum layer. Hence the distribution of the gold particles cannot be visualized. Our aim was to modify the existing embedding methods of cells in such way that no protective platinum layer is needed when cells are being sectioned and viewed in the dual beam microscope. The developed extreme layer plastification method allowed us to both visualize the distribution of the gold particles on the cell surface and the interior of the cell. Moreover, after viewing cells with a light-microscope we successfully applied the ETLP method to visualize the same selected cell in the dual-beam. In addition, we were also successful when we practiced the ETLP method to investigate plant pathogenic fungi with a dual-beam microscope, and different organelles at the site of the fungal cross - walls could be revealed.

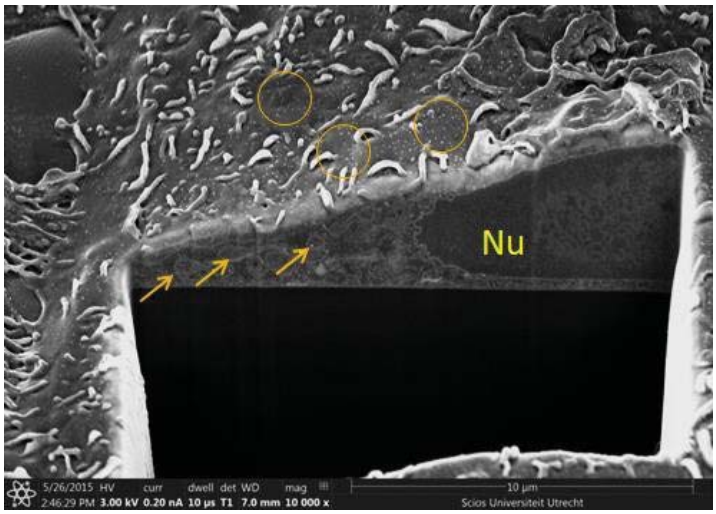
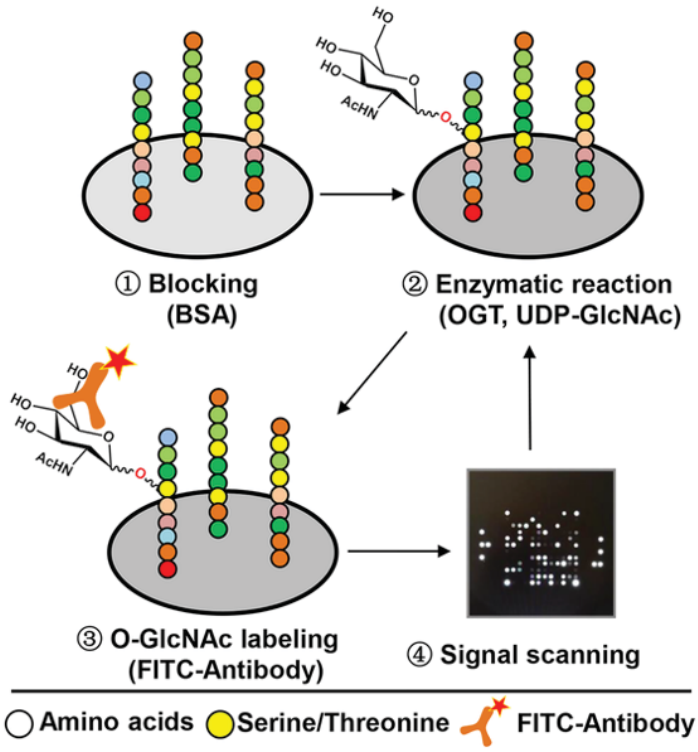


Figure: In a selected HER2 immuno-gold labelled human breast cancer SKBR3 cell a front ditch has been milled with the focused ion beam. After scanning electron microscopy in backscatter mode the distribution of the HER2 immuno-gold labelling as bright spots (circles) on the cell surface and the intracellular organization with mitochondria (arrows) and a part of the nucleus (Nu) can clearly be seen.

Roland Pieters

O-GlcNAcylation is a reversible and dynamic protein post-translational modification in mammalian cells. The O-GlcNAc cycle is catalyzed by O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). O-GlcNAcylation plays important role in many vital cellular events including transcription, cell cycle regulation, stress response and protein degradation, and altered O-GlcNAcylation has long been implicated in cancer, diabetes and neurodegenerative diseases. Recently numerous approaches have been developed to identify OGT substrates and study their function, but there is still a strong demand for highly efficient techniques. In collaboration with PamGene (Rob Ruijtenbeek), Jie Shi and Suhela Sharif in the lab are exploring the use of a microarray to visualize GlcNAcylation and the opposite reaction, i.e. de-GlcNAcylation. They already demonstrated the utility of a peptide microarray approach to discover novel OGT substrates and study its specificity. As a first step peptides were chosen derived from kinase substrates as phosphorylation and O-GlcNAcylation are correlated in mysterious ways. Furthermore, peptides derived from less likely protein substrates were also tested in the assay. Interestingly, a peptide derived from a protein was found as a substrate which is a key regulator of entry into cell division and may function as a tumor suppressor. This approach will likely prove useful for the discovery of novel proteins involved in O-GlcNAc transfer and facilitate interference with these processes as well.



Professor Pieters studied organic chemistry at the University of Groningen, the Netherlands (MSc. 1990) where he worked with Prof. Ben Feringa and also as an exchange student at Trinity University in San Antonio. He completed his Ph.D. at MIT with Prof. Julius Rebek Jr. in 1995 and was an NWO Talent post-doctoral at the ETH-Zürich with Prof. Francois Diederich. After another post doctoral stay (University of Groningen) he joined Utrecht University as an assistant professor in 1998 and obtained a fellowship from the Royal Netherlands Academy of Arts and Sciences and coordinated the EU project POLYCARB. He became an associate professor in 2005, obtained a VICI grant in 2008, and was promoted to full professor in 2010. His current research interests at the department of Chemical Biology and Drug Discovery are directed towards glycodrugs, by studying the interference with protein-carbohydrate interactions using multivalent systems of varying architectures including those with rigid spacers, and using fragment libraries, for targets such as viral and bacterial adhesion proteins and toxins, galectins and glycosidases, Furthermore his group uses glyco- and peptide-microarrays in chemical biology and drug discovery e.g. on O-GlcNAcylation.

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Selected publications

Pukin, A.V. et al. Thiourea-based spacers in potent divalent inhibitors of Pseudomonas aeruginosa virulence lectin LecA. J. Org. Biomol. Chem. 13:10923-10928 (2015)

Visini, R. et al. Structural Insight into Multivalent Galactoside Binding to Pseudomonas aeruginosa lectin LecA. ACS Chemical Biology 10:2455-2462 (2015)

Fu, O. et al. Tetra- versus Pentavalent Inhibitors of Cholera Toxin. Chemistry Open 4:471-477 (2015)

Holger Rehmann

Associate Member

Holger Rehmann studied biochemistry at the Ruhr Universität in Bochum, Germany, where he obtained his Diplom in 1999. He joined the group of Alfred Wittinghofer at the Max-Planck-Institut für Molekulare Physiology in Dortmund, Germany, and defended his Ph.D. thesis entitled "Regulation of Epac by cAMP" at the University of Utrecht, The Netherlands, with distinction in 2003. He performed postdoctoral work in the laboratory of Johannes L. Bos at the University Medical Centre Utrecht, where he establish a structural orientated research line and where he is appointed as Assistant Professor since 2008. He obtained the Otto-Hahn-Medaille of the Max-Planck-Gesellschaft in 2004 and the Hendrik Casimir-Karl Ziegler-Forschungspreis of the Nordrhein-Westfälische Akademie der Wissenschaften and the Koninklijke Nederlandse Akademie van Wetenschappen in 2007 and was veni lauredant in 2007. His research interest is the structural understanding of regulatory events occuring in cellular singnalling pathways.

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Selected publications

Popovic, M. et al. The structure of the Guanine Nucleotide Exchange Factor Rlf in complex with the small G-protein Ral identifies conformational intermediates of the exchange reaction and the basis for the selectivity. J. Struct. Biol. in press (2016)

Schwede, F. et al. Structure-guided design of selective Epac1 and Epac2 agonists. PLOS Biology 13:e1002038 (2015)

We are interested in the structural basis of molecular regulation mechanisms in cellular signalling and in the selectivity of protein-protein and protein-ligand interactions. One focus is on small G-protein, which form a family of about 150 members and act as central switches in many signalling pathways.

We have developed a protocol that allows us to precipitate interactors from cell lysates with recombinant G-proteins. Subsequently proteins are identified by mass spectroscopy in a single run per precipitation condition. Interactors are identified with high confidence by an unbiased data processing routine. Novel interactors are identified and a comprehensive interactome of G-proteins is displayed. At present data from 70 G-proteins were obtained and the data set will still be expanded. Precipitations are performed from tissue culture lysates as well as from tissue preparations.

Identified novel interactors are subjected to detailed biophysical and biological studies. Recombinant proteins are used to study the nature of the interaction, which allows to classify the identified interactors as regulators of the G-protein cycle or as effectors that mediate downstream signalling. Molecular insight into the regulation mechanism are obtained from structural studies. The physiological significance of the interactions is addressed in model cell systems.

We hope to discover many interesting links related to G-protein signalling. For example, our data suggest a so far unrecognised link of the small G-protein Rab24 to the regulation of mitochondrial fusion and fission. This process plays an important role in maintaining the proper organelle structure of cells. Another link might connect the small G-protein Rerg, which function is largely unknown, to the regulation of the cytoskeleton.

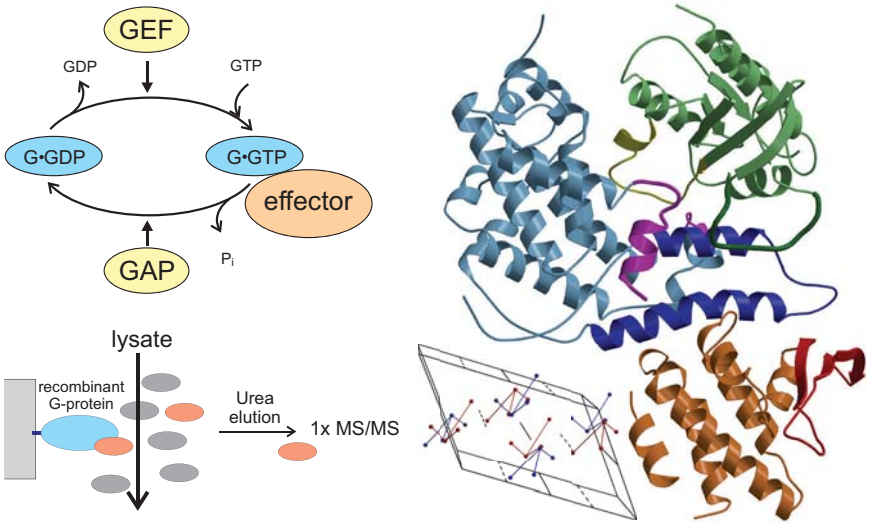

Figure: Small G-proteins act as molecular switches that cycle between a GDP and a GTP bound conformation. Novel G-protein interacting proteins, which are either involved in regulating the switching process or in transmitting the signal, are identified in an approach based on mass spectroscopy. The right panel shows the crystal structure of the small G-protein Ral in complex with its regulator Rlf. Features of the crystal lattice are depicted in the insert.

Stefan Rüdiger

Protein damage is a key problem in the life sciences and molecular medicine. There is no cure for related diseases – such as Alzheimer, Parkinson or ALS, but also many other diseases with dramatic impact for the affected individual such as ataxias or cystic fibrosis. These diseases have in common that the molecular cause is related to uncontrolled consequences of protein damage and aggregation. Our body is not unprotected against protein damage. The fidelity of protein shape in the cell is maintained by a powerful proteostasis network, in which molecular chaperones and proteases play a key role. We have recently described a structural model of a complex of the molecular chaperone Hsp90 with the Tau protein, which aggregates in Alzheimer. We found Hsp90 to bind to the Tau's microtubule-binding repeat region, which forms the toxic aggregates. Together this suggests a direct role for Hsp90 in dealing with Tau aggregation. We hypothesize that molecular chaperones typically counter aggregation of Tau, but a decrease in chaperoning capacity at higher age may allow fatal aggregation to proceed. Our aim is now to test the function of the natural defence system in the origin of Alzheimer Disease.

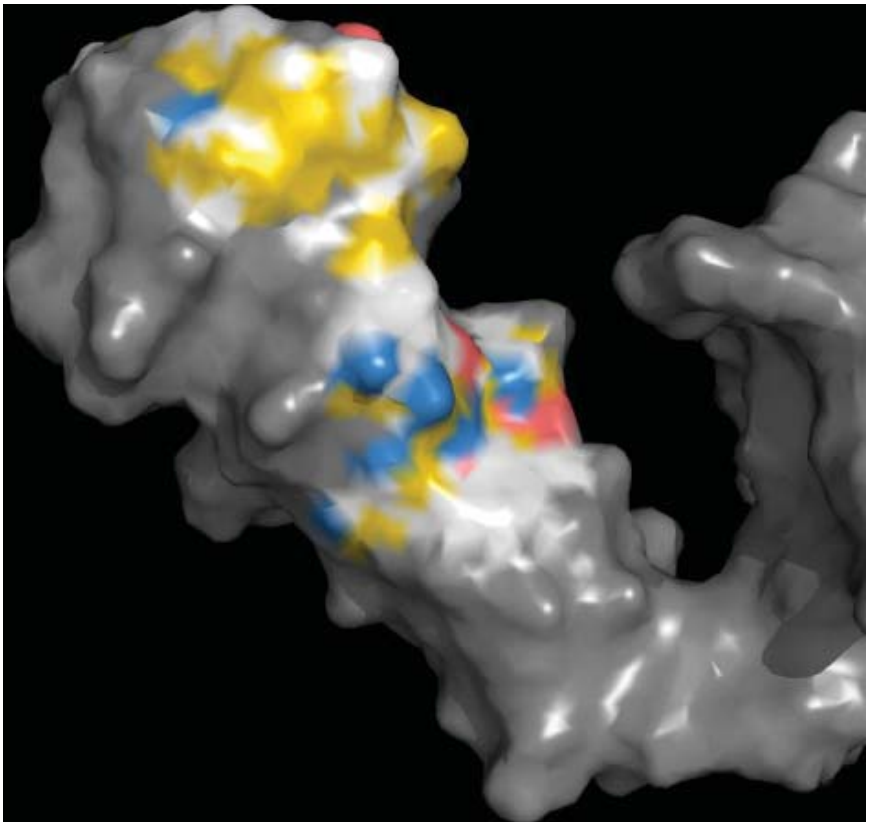

Figure: The substrate binding site of the molecular chaperone extends over a large region of the molecule. It consists of hydrophobic patches (yellow) and scattered charges (blue, positive charges and red, negative charges). Colours according to Hagemans et al., Frontiers Biosci. 2015)

Stefan Rüdiger graduated in Chemistry at Heidelberg University, Germany. Afterwards he joined Bernd Bukau's laboratory and in 2000 he obtained the PhD at Freiburg University, Germany. After a postdoc in Alan Fersht's laboratory at the Centre for Protein Engineering, Cambridge University and MRC Centre, Cambridge, UK, he became in 2004 faculty member at the Bijvoet Center of Utrecht University, The Netherlands. In 2006 he received tenure and became Marie Curie Excellence Team Leader and High Potential of Utrecht University. In 2012 he became the coordinator of the first ever awarded Marie Curie Innovative Doctoral Programme in the Life Sciences, ManiFold.

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Selected publications

Hagemans, D. et al. A script to highlight hydrophobicity and charge on protein surfaces. Frontiers Mol Biosci 2:56 (2015)

Karagöz, G.E. et al. Hsp90 interaction with clients. TIBS 40:117-125 (2015)

Karagöz, G.E. et al. Hsp90-Tau complex reveals molecular basis for specificity in chaperone action. Cell 156:963-974 (2014)

Richard Scheltema

Richard Scheltema (b. 1975, University of Utrecht, >20 papers, h-index 14, formerly MPI for Biochemistry) is an expert in high performance LC-MS/MS technology and pioneered several bioinformatics approaches for raw mass spectrometry data acquisition, extraction and down-stream analysis for both metabolomics and proteomics experiments. Most recently he developed a novel chemical crosslinking approach by mass spectrometry and an ultra-high throughput interaction proteomics LC-MS/MS platform. His current focus is on structural biology. He has been involved in investigations into various facets of the immune response and inflammation. He is currently heading an independent group within the broader context of the Heck laboratory working on instrument developments with a special interest in chemical crosslinking mass spectrometry applied to membrane proteins and the extra cellular matrix. In 2015 he was awarded an UIPS seed grant for the development of novel crosslink chemicals.

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Selected publications

Fort, KL. et al. Implementation of Ultraviolet Photodissociation on a Benchtop Q Exactive Mass Spectrometer and Its Application to Phosphoproteomics. Anal Chem. 88:2303-10 (2016)

Hosp, F. et al. A Double-Barrel Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) System to Quantify 96 Interactomes per Day. Mol Cell Proteomics. 14:2030-41 (2015)

Benda, C. et al. Structural model of a CRISPR RNA-silencing complex reveals the RNA-target cleavage activity in Cmr4. Mol Cell. 56:43-54 (2014)

Extracellular sensing and communication is arranged by a wide array of receptors at the cell surface. Each receptor is capable of binding ligands, eliciting an appropriate response from the cell. Even though the receptors consist of a single protein, woven into the cell membrane, they rely on other proteins for proper function. In the case of the well-known pattern recognition receptor (PRR) TLR4, for which we studied protein secretion upon LPS sensing, 3 additional proteins are involved that together with TLR4 build up a complex (Fig. 2A). The receptor itself is built up out of a TLR4 dimer interacting with CD14. A protein LBP first binds the LPS molecule, which enhances the affinity of LPS to CD14. After successful binding to CD14 the LPS is transferred to TLR4 and bound with the help of MD2, upon which signal transduction inside the cell is initiated.

The relatively low complexity found for the secretome, compared to whole cellular lysates, invites to perform more elaborate experiments than only studying the secreted proteins individually. To illustrate, with current mass spectrometry technology (for example the Orbitrap Q Exactive HF) whole cell lysates consisting of 10,000 proteins can almost be fully analyzed within a single day. My group is setting out to use this low complexity to unbiasedly chart the assembly and disassembly of extracellular protein machinery plus the complement of secreted proteins upon a given stimulus. To achieve this goal a combination of chemical crosslinking techniques are used to capture receptors still embedded in the membrane to retain their shape and function up to lysis and various enrichment techniques for membrane associated proteins.

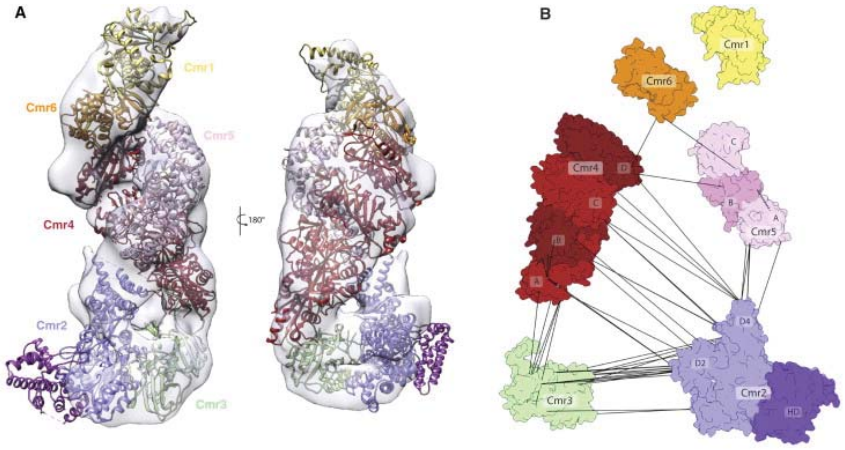


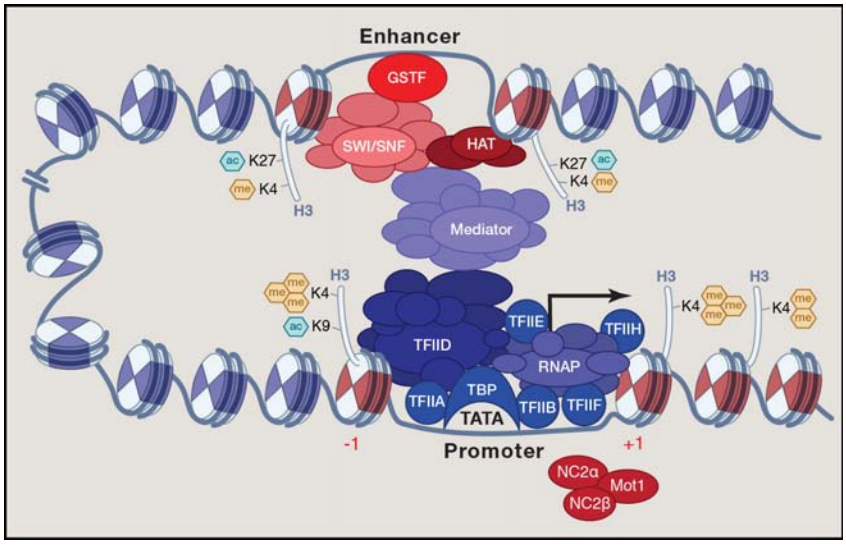
Figure: *Pseudoatomic Model of the Cmr Complex.* (A) View of two orientations of the resolution cryo-EM map of the Pf Cmr complex (EMD-5740) fitted with the atomic models of the individual Cmr proteins. Molecules are color coded as in Figure 1. The small, unaccounted density is near the N terminus of Cmr6ΔN. (B) Cmr complex topology represented by interprotein crosslinks (Cα-Cα distances < 35 Å) and simplified representations of the Cmr building blocks. For visibility, the model is shown in an exploded view. Multiple crosslinks between Cmr2 (D2 and D4) and Cmr4 or Cmr5 demonstrate the close interaction and relative orientation of these subunits. Crosslinks from Cmr3 to Cmr4 outline a binding epitope for Cmr4. Interprotein links between identical proteins (Cmr4-Cmr4 and Cmr5-Cmr5) are shown as dashed lines. Only one crosslink was found between Cmr4 and Cmr5, Cmr6 and Cmr4, and Cmr6 and Cmr5. Cmr1 was omitted in the CXMS experiment.

Marc Timmers

Regulation of transcription and chromatin is the general research theme of the Timmers group. The emphasis is on the basal transcription machinery with a focus on TFIID. The essential TFIID transcription factor is central in the assembly of RNA polymerase II transcription complexes and controlled by a variety of mechanisms including chromatin modifications. A key chromatin modification in this is methylation of histone H3 at lysine-4 (H3K4), which we have shown to anchor TFIID to gene promoters.

Methylation mark at H3K4 in human cells occurs by the six SET1/MLL complexes. The catalytic center of these large macromolecular complexes is formed by a SET1A/B or MLL1-4 subunit, which is frequently mutated in a variety of human cancers or in development disorders. For example, heterozygous inactivation of the MEN1 gene, encoding the menin subunit of MLL1/MLL2, represents the molecular basis for multiple endocrine neoplasia type 1 (MEN1), which affects ~400 individuals in the Netherlands.

Our previous work revealed that the menin subunit acts as a molecular adaptor in the recruitment of the MLL1/2 histone methyltransferase complexes to activated hormone receptors like estrogen receptor-α, the vitamin D receptor and PPAR-γ. These findings prompted us to investigate the occurrence of breast cancer in Dutch MEN1 families. In a close collaboration with endocrinologists, surgeons and cancer pathologists of the UMCU we discovered that the MEN1 gene is a breast cancer susceptibility gene. Female MEN1 patients have 25-30% risk to develop breast cancers in their life times, which represent at an average age of 48 years contrasting diagnosis at 60 years in the general population. These same characteristics were observed in French, New England and Tasmanian MEN1 cohorts. These findings form the basis for early breast cancer detection programs in MEN1 families and attracted the attention of the (inter) national news media.



Marc Timmers studied gene regulation through a post-doc (1990-1992) in the lab of Phillip Sharp (Nobel laureate Medicine 1993) at the MIT and a Ph.D. (1985-1990) with Alex van der Eb at the Leiden University. He obtained his M.Sc. 'cum laude' in chemistry at the Leiden and Amsterdam (UvA) universities. In 1992 he started his independent research as a KNAW fellow in the Laboratory of Physiological Chemistry of the Utrecht University. Since 2001 Marc Timmers is a full professor of Epigenetics and Gene Regulation at the University Medical Center Utrecht in the department of Molecular Cancer Research of the Center for Molecular Medicine.

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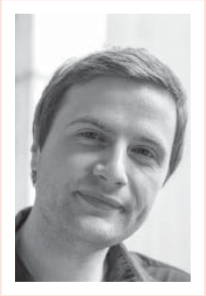
Selected publications

Koster, M.J.E. et al. Genesis of chromatin and TBP dynamics in the origin of species Cell 161:724-36 (2015)

Koster, M.J.E. et al. Regulation of antisense transcription by Mot1p and NC2 via removal of TATA-binding protein (TBP) from the 3'-end of genes Nucleic Acids Res. 43:143-52 (2015)

Dreijerink, K.M.A. et al. Breast cancer predisposition in Multiple Endocrine Neoplasia type 1 (MEN1) New England Journal of Medicine 371:583-4 (2014)

Markus Weingarth



Markus Weingarth (1982) completed his Ph.D in 2010 with Geoffrey Bodenhausen at the Ecole Normale Supérieure, working on NMR pulse sequence development. He then started a post-doc with Marc Baldus at Utrecht University, first as FEBS fellow (2010) and later on with a VENI grant (2012), working on supramolecular studies of membrane proteins using solid-state NMR and computer simulations. He was awarded 'FEBS Young Distinguished Scientist' for his post-doc studies (2014). His current research, which is supported by a VIDI grant (2015), focuses on the development of highly sensitive solid-state NMR methods to study challenging biological systems such as membrane proteins in native cellular environments.

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Selected publications

Mance, D. et al., A labeling approach to harness backbone and side chain protons in 1H-detected solid-state NMR, Angew Chem, 54:15799 (2015)

Rad Malekshahi, M. et al., The supramolecular organization of a peptide based nanocarrier at high molecular detail, J Am Chem Soc, 137:7775 (2015)

Sinnige, T. et al., Proton clouds to measure non-exchangeable sidechain protons in solid-state NMR, J Am Chem Soc, 136:4452, Cover article, (2014)

Our works focuses on the development of proton-detected solid-state NMR (ssNMR) methods, which can greatly increase spectral ssNMR sensitivity by much more than one order of magnitude. We also use these ultra-sensitive methods to study very challenging systems such as heterogeneous peptide assemblies or membrane proteins in native cell membranes. Without ^{1}H-detection, such studies would be hardly possible or much curtailed.

However, ^{1}H-detection in ssNMR is yet an unfledged technique and, in particular, not much suitable for membrane proteins. This is because ^{1}H-detection requires protein expression in fully deuterated media, together with subsequent ^{1}H/^{2}D back-exchange in protonated buffer. Obviously, only exchangeable protons can be detected in this manner, which means three paramount limitations for ^{1}H-detections: 1) >95 % of the side-chains, which have only non-exchangeable protons, cannot be investigated with 1H-detection. 2) Membrane proteins cannot be thoroughly studied, given that their transmembrane parts are lipid-shielded, thus do not back-exchange and remain invisible for ^{1}H-detected methods. 3) Moreover, another substantial worry is that such ^{1}H-detected methods cannot be applied with mammalian or insect cells, which do not express in highly deuterated solvents. We could recently provide solutions to all of these problems. For example, we have introduced a new method for ^{1}H-detection dubbed 'fractional deuteration', which allows harvesting very well resolved side-chain protons for structural studies (see Figure below) and this method was also selected as 'Highlight' by the NWO. Recently, we have even successfully managed to acquire very well resolved ^{1}H-detected spectra of membrane proteins '*in cellulo*', i.e., directly in a native bacterial membrane without protein purification.

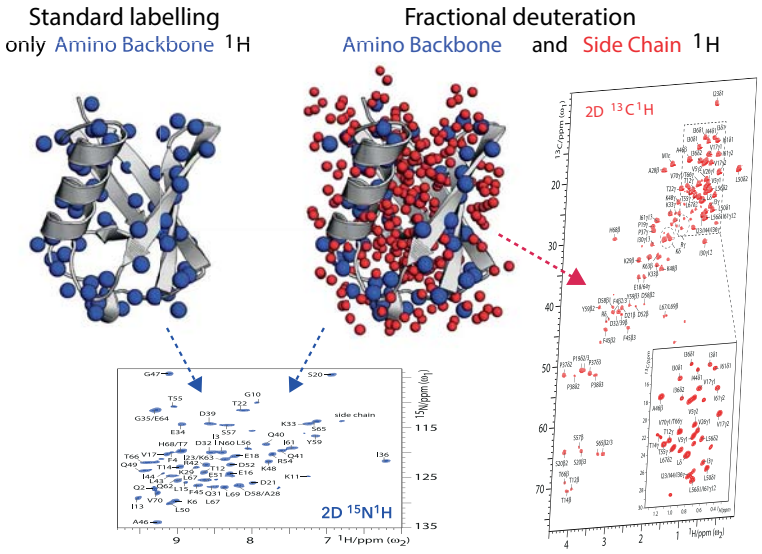

Figure: Fractional Deuteration (Mance et al., Angew. Chem. 2015) allows for the direct detection very well resolved side-chain protons in solid-state NMR.